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Immobilization of 2,4- and 2,6-Dinitrotoluenes in Soils and Compost

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Final report

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Preface

This report was prepared by the Environmental Laboratory (EL), Vicksburg, MS, of the U.S. Army Engineer Research and Development Center (ERDC), in partnership with the U.S. Geological Survey (USGS), Denver, CO, and DynTel, Vicksburg, MS. The research was sponsored by BT25, Environmental Quality Basic Research Program, Washington, DC, Dr. M. John Cullinane, Program Manager, under project Work Unit BR-202. The Principal Investigator was Dr. Judith C. Pennington, EL; Co-Principal Investigator was Dr. Kevin Thorn, USGS.

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1   Introduction

The dinitrotoluenes, 2,4-dinitrotoluene (2,4-DNT) and 2,6-dinitrotoluene (2,6-DNT), are the major impurities of 2,4,6-trinitrotoluene (TNT), and are usually present wherever soils have been contaminated with TNT (Nishino et al. 1999; Spanggord et al. 1991; Hughes, Wang, and Zhang 1999). The DNTs are also a major component of propellants for artillery shells, and can be found as contaminants in soils on firing ranges in the immediate vicinity of firing points (Pennington et al. 2001). Windrow composting has been used to remediate soils contaminated with the explosives TNT and 1,3,5-hexahydro-1,3,5-trinitrotriazine (RDX) at several Army munitions production sites within the United States (Alleman and Leeson 1997, 1999). An assessment of the efficacy of windrow composting should therefore address the fate of the DNTs during bioremediation.

Previous reports described the use of ^{15}N nuclear magnetic resonance (NMR) spectroscopy to gain a molecular-level understanding of the reduction and binding of TNT during composting (Pennington et al. 1998, 1999; Thorn 1997, 1998; Thorn and Kennedy 2002; Thorn, Pettigrew, and Goldenberg 1996; Thorn, Pennington, and Hayes 2001, 2002; Thorn et al. 1996, 1999). Nitrogen-15 NMR analysis revealed how the major reductive degradation products of TNT (2-amino-4,6-dinitrotoluene (2ADNT); 4-amino-2,6-dinitrotoluene (4ADNT); 2,4-diamino-6-nitrotoluene (2,4DANT); 2,6-diamino-4-nitrotoluene (2,6DANT); 2,4,6-triaminotoluene (TAT)) formed covalent bonds with soil humic acid, ligno-cellulose, and model lignin and quinone compounds, in the presence and absence of the phenoloxidase enzyme and metal catalysts, horseradish peroxidase (HRP), mushroom tyrosinase, and birnessite. All five amines underwent nucleophilic addition reactions with quinone and other carbonyl groups in the humic acid to form heterocyclic and nonheterocyclic condensation products. The ^{15}N NMR analyses revealed differences in the reactivity of the amines. For example, whereas the diamines and TAT readily undergo 1,2-nucleophilic addition with quinone groups to form imines, the monoamines form imines only to a limited extent. The reduction and binding of 2,4,6-trinitrotoluene-^{15}N$_3$ (T^{15}NT) in an aerobic bench-scale reactor simulating the conditions of windrow composting were also studied by ^{15}N NMR. In general, the types of bonds that formed between soil organic matter and reduced TNT amines in controlled laboratory reactions were observed in the spectra of the whole compost and fractions. These results confirmed that during composting, TNT is reduced to amines that form covalent bonds with organic matter through aminohydroquinone, aminoquinone, heterocyclic, and imine linkages, among others. The concentrations of imine nitrogens in the compost spectra suggest that covalent binding by the diamines,

2,4DANT and 2,6DANT, is a significant process in the transformation of TNT into bound residues.

This report discusses the extension of the TNT studies to the DNTs, 2,4-DNT and 2,6-DNT. The major reductive degradation product of 2,4-DNT, 4-methyl-3-nitroaniline, labeled with ^{15}N in the amine position, was reacted with soil humic acid in the presence and absence of HRP. The ^{15}N-labeled DNTs were subjected to incubation in the aerobic reactors and the finished composts analyzed by ^{15}N NMR. The fulvic acid, humic acid, humin, and lignocellulose fractions isolated from the 2,4-DNT compost were also analyzed by ^{15}N NMR. Aniline, nitro-benzene, and ammonium nitrate, all labeled with ^{15}N, were also subjected to the aerobic composting. The accumulated analyses allowed a final comparison of the whole composts of TNT, 2,4-DNT, 2,6-DNT, aniline, nitrobenzene, and ammonium nitrate by solid-state cross-polarization/magic angle spinning (CP/MAS) ^{15}N NMR.

Several studies have examined the biodegradation of 2,4- and 2,6-DNT (Lendenmann, Spain, and Smets 1998; Liu, Thomson, and Anderson 1984; Hughes, Wang, and Zhang 1999; McCormick, Cornell, and Kaplan 1978; Nishino et al. 1999; Spanggord et al. 1991; Haigler, Nishino, and Spain 1994; Cheng et al. 1996). Reports suggest that under aerobic conditions, the DNTs are reduced to the monoamine stage, but not the diamine stage (McCormick, Cornell, and Kaplan 1978). Complete reduction to the diamines has been observed under anaerobic conditions (McCormick, Feeherry, and Levinson 1976). The reduction pathways of 2,4- and 2,6-DNT proposed by Hughes, Wang, and Zhang (1999) and others (McCormick, Cornell, and Kaplan 1978) are illustrated in Figure 1. The potential number of products formed from the reduction of the DNTs that can subsequently undergo covalent binding with organic matter should be less than in the case of TNT.

Rationale

Covalent bonding of amino transformation products of TNT to functional groups on humic acid results in immobilized products that are not hydrolyzable by acids or bases, microbially degradable, or leachable (Pennington et al. 1999). However, the extent to which these reactions apply to DNTs is unknown. Since DNTs are considered toxic and many explosives-contaminated sites exhibit DNTs as well as TNT, the fate of DNTs is relevant to remediation and risk assessment. Some sites, e.g., Badger Army Ammunition Plant and Massachusetts Military Reservation, have significant contamination by DNTs alone. Imple-mentation of remedies such as composting DNT-contaminated soils and moni-tored natural attenuation of groundwater requires an understanding of the potential for formation of covalently bonded immobilization products. Further-more, the extent of immobilization is directly related to exposure potential of environmental and human receptors.

Reactions of 2,4- and 2,6-DNTs may differ significantly from behavior of amino transformation products of TNT. For example under aerobic conditions, DNTs are reduced to monoaminodinitrotoluenes, but not to diaminonitrotoluenes

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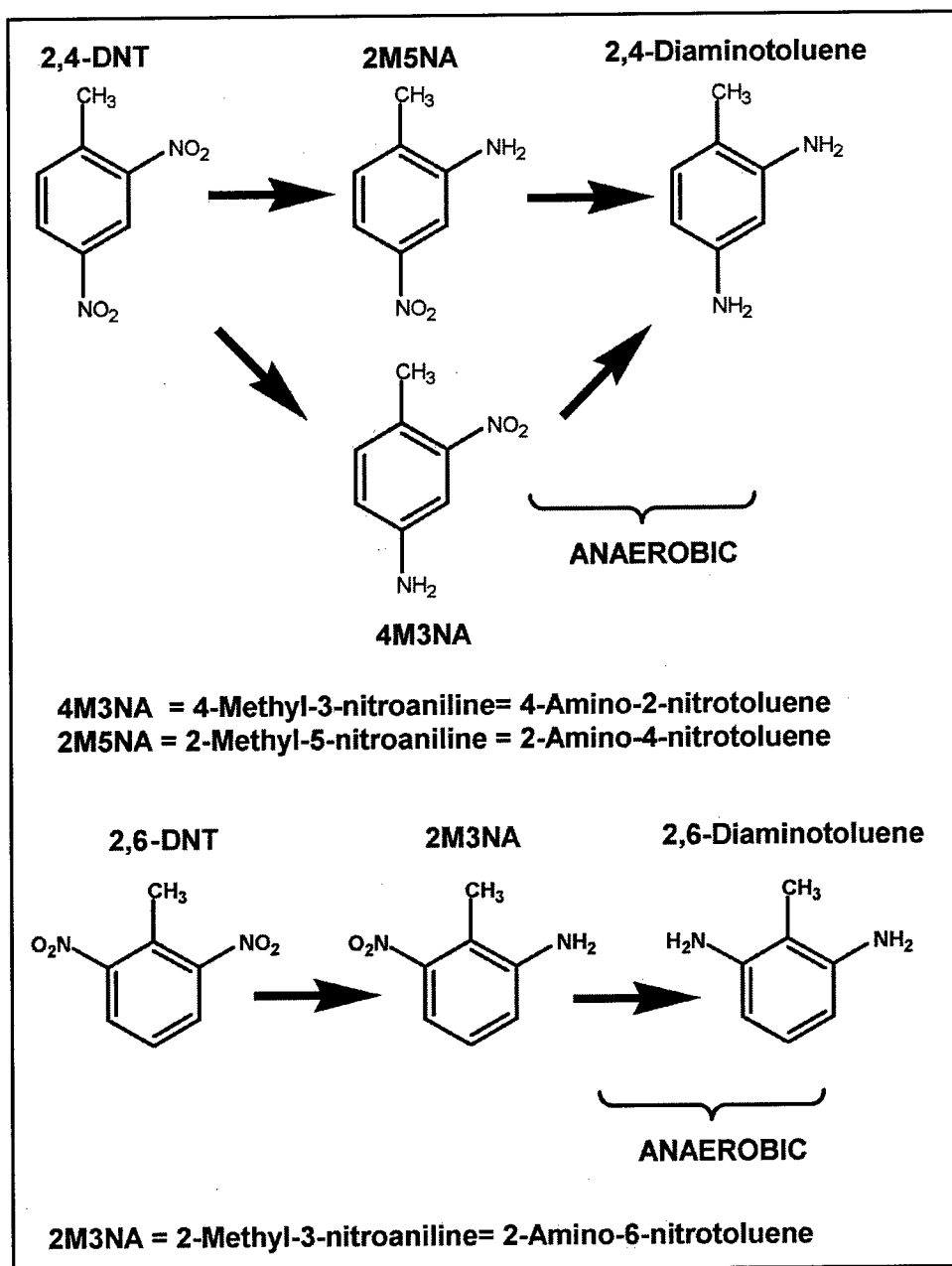
Figure 1. Reduction pathways for 2,4- and 2,6-DNT. Nitro groups are reduced to amines via nitroso and hydroxylamine intermediates

(McCormick, Feeherry, and Levinson 1976; Rieger and Knackmuss 1995; McCormick, Cornell, and Kaplan 1978; Liu, Thomson, and Anderson 1984). Furthermore, nitrite elimination from 2,4-DNT transformation products results in a nitrated catechol; i.e., one nitro group remained unreduced through initial steps in microbial degradation (Spanggord et al. 1991; Haigler, Nishino, and Spain 1994). Typically under aerobic conditions, mononitrotoluenes and DNTs are degraded via oxygenase reactions whereas TNT is reduced to amines (Rieger and Knackmuss 1995). Products of oxygenase reactions tend to have hydroxylated (-OH) rather than aminated ($-NH_2$) substitutents. Reactions of these hydroxylated products with humic functional groups are much less likely than reactions of

aminated products. Therefore, the reduction of nitro groups to amines may serve as a limiting process making the DNTs much less subject than TNT to immobilization reactions.

Objectives

The hypothesis of the study is as follows: transformation and subsequent immobilization via formation of covalent bonds to organic matter in soil (and in remediation matrices such as compost) occur and reduce mobility and bioavailability of DNTs, thereby reducing the risk to environmental and human receptors.

This study will demonstrate the potential for reactions in soils and determine the bonding mechanisms of amino transformation products of DNTs to various fractions of organic matter. Although these reactions have been demonstrated for anilines and for the amino transformation products of TNT, similar reactions for DNTs have not been investigated. Use of ^{14}C mass balance in soils coupled with high-performance liquid chromatography (HPLC) analysis of partitioned soil extracts will quantify soil partitioning, the aqueous and solvent unextractable residuals, and the extent of transformation to amino and other products in soils. These results coupled with ^{15}N NMR analyses of the products of reactions between transformation products and surrogate functional groups, compost fractions, and whole compost provide a unique approach for observing reactions, defining the chemical reaction mechanism(s), and identifying the potential products. Long-term geochemical stability of products may be inferred from the nature of the products and bonds produced. The following are specific objectives:

a. To determine the extent to which DNTs and/or their amino transformation products are immobilized in soils.

b. To demonstrate the mechanisms of reactions between aminonitrotoluenes and functional groups of soil organic matter and compost.

2 Materials and Methods

Interactions with Soils and Compost

Adsorption kinetics

To determine the rate at which the DNTs are adsorbed by soils, adsorption kinetics tests were conducted with three soils exhibiting a range in total organic carbon (TOC), cation exchange capacity (CEC), and particle size distribution. Grange Hall and Yokena Clay soils were collected near Vicksburg, MS, the Picatinny soil was from Picatinny Arsenal, NJ, and the Fort Lewis soil was from Fort Lewis, WA. Characterization of soils included determinations of the following: particle size distribution (Day 1956 as modified by Patrick 1958), CEC as determined by the ammonium saturation method (Plumb 1981) and analyzed by U.S. Environmental Protection Agency (USEPA) Standard Method 350.1 (USEPA 1982), and TOC, by the American Public Health Association (APHA) Method 5310D (APHA 1989) (Table 1). Soils were prepared by air-drying and sieving (Number 10 sieve, 1.65 mm) to remove any coarse gravel. Adsorption kinetics were determined in triplicate by amending soils with 10 mg L^{-1} $[U-^{14}C]$ 2,4-DNT or $[U-^{14}C]$ 2,6-DNT in a 4:1 solution to soil ratio in separate tests and equilibrating on a reciprocating shaker for the following times: 0, 1, 12, 24 hr, and 3 and 5 days.[1] One milliliter of the solution phase was assayed by liquid scintillation (LS) counting after separation by centrifugation (15 min at 9,400 relative centrifugal force (RCF). The solution phase was also analyzed by HPLC. Solution phase concentrations were plotted over time to create a kinetics curve.

Table 1 Properties of Test Soils					
	Particle Size Distribution, percent				
Soil	Sand	Silt	Clay	CEC, mmol g-1	TOC, %
Grange Hall	39	51	10	16.7	0.29
Yokena Clay	14	37	49	38.9	2.4
Picatinny	62.5	32.5	5	9.8	0.63
Fort Lewis	63.2	17.5	19.3	4.7	11.3

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[1] Specific activities of 2,4-DNT and 2,6-DNT were 16.82 and 18.38 mCi mmol^{-1}, respectively; radiochemical purity of each was 99 percent by HPLC.

Partition coefficients

Soil partition coefficients describe the affinity the compound has for adsorption to soils. Partition coefficients are useful in predicting compound leaching potential, subsurface transport potential, and bioavailability. Three replicates of each of the three soils were partitioned with aqueous solutions of either radiolabeled 2,4-DNT or 2,6-DNT at 0.10, 0.50, 1.0, 5.0, and 10.0 mg kg^{-1}. After equilibration for the adsorption steady-state period (determined by results of adsorption kinetics experiments), the solution phase was assayed by LS counting as described previously. A linear model was applied to solution phase concentrations regressed against soil phase concentrations to determine partition coefficients, the slope of each regression line.

Desorption kinetics

The rate at which the DNTs desorb from soil contaminated in the field provides an indication of the mobility of the contaminant. Three replicates of a soil collected near the muzzle of a 105-mm howitzer at Fort Lewis, WA, were used to determine desorption kinetics (Table 1). The soil was contaminated with 98.5 mg kg^{-1} 2,4-DNT (no other analytes were detected), which is a component of the propellant used to fire the round. Each replicate was desorbed with distilled deionized water for each of the following times: 30 min, 1, 2, 5, and 12 hr. The aqueous phase was analyzed by HPLC. Values were plotted against time to develop a desorption kinetic curve.

Sequential desorption

Another indicator of mobility potential of a compound in soil is the sequential desorption isotherm. This isotherm provides an estimate of leaching potential from sequential rainfall events. Three replicates of the soil from Fort Lewis were subjected to three sequential desorption challenges with distilled deionized water. After each 12-hr equilibration, 1 mL of the aqueous phase was assayed by HPLC, the aqueous phase was removed, and a fresh aqueous phase added. To determine a desorption coefficient, solution phase concentrations were plotted against soil phase concentrations as described previously for partition coefficient plots. Solution phase concentrations for each cycle were compared by analysis of variance (ANOVA) to determine whether differences were sufficient to constitute a meaningful desorption isotherm.

Soil fractionation

The soil having the highest organic carbon content, Yokena clay, was equilibrated with 100 mg kg^{-1} of each radiolabeled compound in three replicates. The soil phase was subjected to fractionation according to procedures used to fractionate compost (see next section). Solution fractions were assayed by LS counting. The solid fractions were subjected to complete combustion in which the carbon dioxide generated was trapped and assayed by LS counting. Mass balance was obtained by summing recovered radioactivity from each fraction.

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Data were analyzed by a one-way ANOVA. Differences in recoveries were compared using the Tukey Test (Steel and Torrie 1980).

Compost Preparation

Composting process

All composts were prepared according to procedures described in Pennington et al. (1995). In brief, Grange Hall soil (0.29 percent organic carbon) was spiked with DNT in an aqueous slurry (4:1 water to soil). The soil slurry was added to a compost mixture and composed 10 percent of the compost wet weight. The compost mixture consisted of 33 percent green cow manure, 22 percent alfalfa, 6 percent chopped apples, 22 percent sawdust, and 17 percent chopped potatoes. The combined soil slurry and compost mixtures were added to reactors that consisted of wide-mouthed glass canning jars (473 cm^3) with modified lids. The lids were fitted with ports for intake of air to the reactor floor, and exit air vented through a series of traps. For radiolabeled tests, air exiting each chamber was passed through a Tenax[1] (2 g) followed by an activated charcoal (5 g) trap to capture volatile organic compounds (VOCs), and 100 ml of 5N KOH to capture carbon dioxide. The carbon dioxide trap was changed every 7 days (3 times) to prevent saturation. The chambers were incubated in a water bath maintained at 55 °C. Airflow was from the bottom at 10 ml min^{-1}. Temperature was monitored automatically by thermocouples in the center of the compost. Compost was incubated for 20 days.

Compost of radiolabeled compounds

Compost was prepared with 100 mg kg^{-1} radiolabeled 2,4- and 2,6-DNT, respectively, in Grange Hall soil. Respective DNTs were added to soil slurries in acetone solution. Tests were stirred 4 to 6 hr in the dark at 5 °C to achieve evaporation of the solvent and homogeneous distribution of the compounds. Slurries were added to compost components as described in the preceding paragraph, and tests were thoroughly mixed. The experimental design consisted of seven replicates and one uncontaminated control for each compound. A sample from each reactor was assayed by complete combustion immediately after loading and at the end of the 20-day incubation. The finished compost was compiled and fractionated to determine the distribution of contaminant/products in the matrix (Pennington et al. 1995). Each fraction, as well as the extracts from each VOC and carbon dioxide trap, was assayed by LS counting. Extracts of the volatile and carbon dioxide traps were also assayed by HPLC (USEPA 1994). Mass balance was determined by the sum of the average percent radioactivity recovered from each fraction. Data were analyzed by a one-way ANOVA. Differences in recoveries were compared using the Tukey Test (Steel and Torrie 1980).

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[1] Altech Association, Inc., Deerfield, IL.

Compost fractionation

Compost was fractionated according to procedures reported in Pennington et al. (1995) (Figure 2). The association of the DNTs with different compost components was examined by extracting the compost with acetonitrile and then fractionating the compost residue into the following functionally defined components: cellulose, humic acid, fulvic acid, and humin (Pennington et al. 1995). Functionally defined components are not structurally defined, but are defined on the basis of their solubility or insolubility in acid or base (or, in the case of cellulose, insolubility in methylisobutylketone, or MIBK). Compounds associated with the aqueous- or solvent-extractable fractions are more readily available to environmental processes such as transport or microbial degradation, while compounds associated with cellulose may become available over time as the cellulose is broken down by endogenous microflora. Cellulose has a half-life of several weeks to years depending upon the local environment. Compounds associated with other fractions are less available. For example, humin is extremely recalcitrant to decomposition, persisting for as long as hundreds of years (Stevenson 1989).
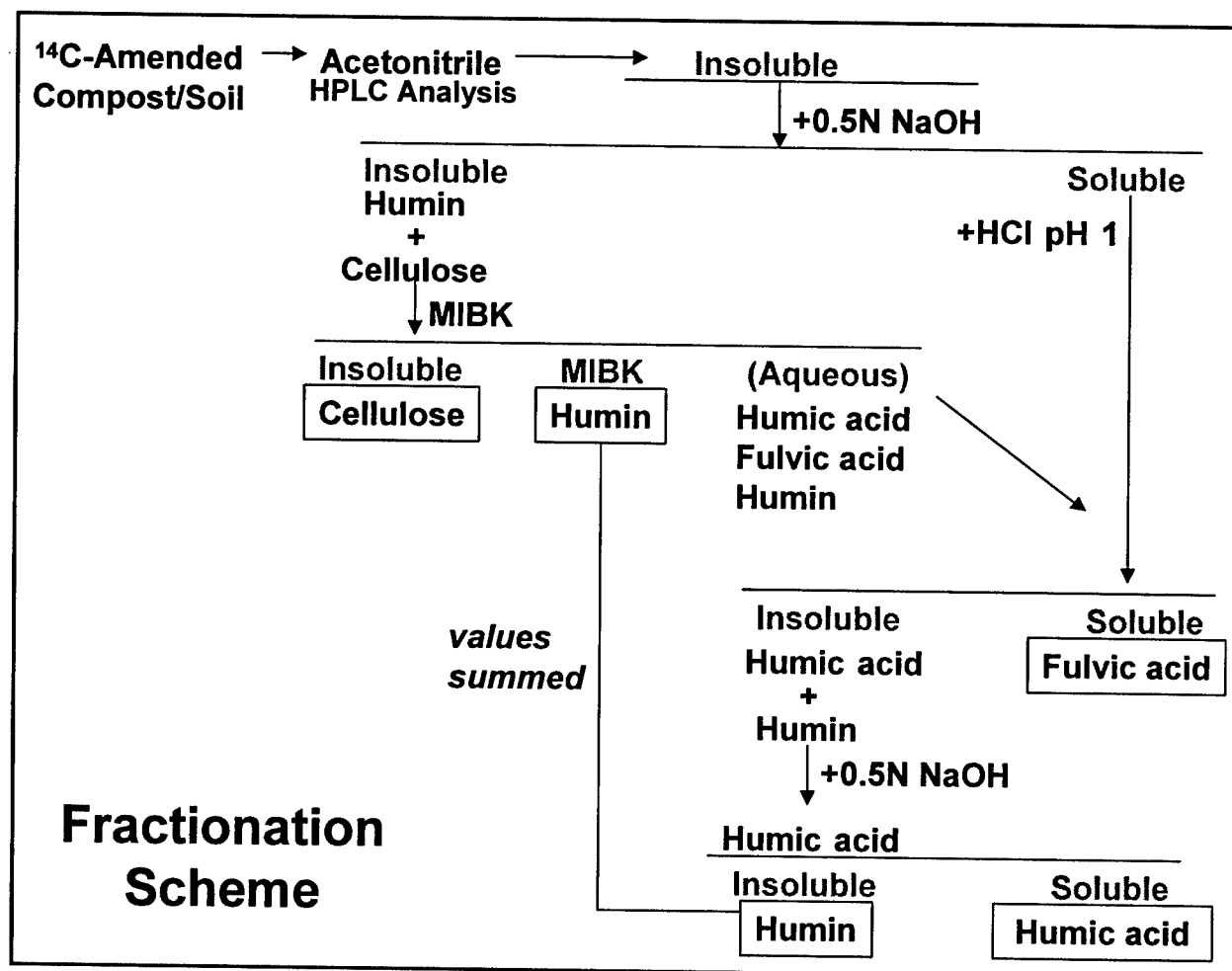

Figure 2. Fractionization scheme for soils and compost

Compost of heavy isotope labeled compounds

To prepare compost for NMR analysis, Grange Hall soil was amended with various ^{15}N-labeled compounds. One compost replicate was prepared as an untreated control, and two replicates each were prepared at 180,000 µg g^{-1} of soil with each of the following ^{15}N-labeled compounds: 2,4-DNT, 2,6-DNT, nitro-benzene, and ammonium nitrate. Two replicates were also prepared with ^{15}N-labeled aniline at 200,000 µg g^{-1} for a total of 11 tests. Although added to soils in acetone solutions, these concentrations exceeded the aqueous solubilities of the compounds. Therefore, as the soil was slurried (4:1, water to soil ratio), precipitation of the compounds resulted in solids in the soil matrix. Slurries were stirred for 4 to 6 hr in the dark at 5 °C to achieve evaporation of the solvent and homogeneous distribution of the compounds. After 20 days of composting, the ^{15}N-labeled 2,4-DNT compost was fractionated and each fraction was assayed by NMR (described in the next section). The remaining composts, except for the ammonium nitrate compost, were analyzed by NMR and HPLC. The ammonium nitrate compost was analyzed by NMR only, since the compost matrix may have contained unlabeled ammonium nitrate from other sources prior to treatment.

NMR Analyses

Materials

The following materials were used in the NMR analyses:

a. The reference soil humic acid (Elliot silt loam soil; Joliet, IL; mollic horizon) was purchased from the International Humic Substances Society (IHSS), *www.ihss.gatech.edu/*.

b. Aniline-^{15}N, nitro-^{15}N-benzene, and $^{15}NH_4{}^{15}NO_3$, all 99 atom percent ^{15}N, were purchased from ISOTEC (Miamisburg, OH).

c. The 4-^{15}N-amino-2-nitrotoluene (proper name = 4-methyl-3-nitro-aniline-^{15}N, hereafter referred to as 4M3NA), 2,4-di(^{15}N)nitrotoluene, and 2,6-di(^{15}N)nitrotoluene, all 99 atom percent ^{15}N, were synthesized by Dr. Ronald Spanggord (SRI, Menlo Park, CA).

d. Horseradish peroxidase (HRP; EC1.11.1.7; 53 purpurogallin units/mg solid) was purchased from Sigma (St. Louis, MO).

Reactions of 4M3NA with soil humic acid

Five hundred milligrams of the soil humic acid was dissolved in 400 mL distilled and deionized water by adjusting the pH to 6.4, followed by addition of 100 mg of the 4M3NA dissolved in 600 mL water. The two solutions were added together, the pH adjusted to 6.0, and the solution stirred for 15 days open to the atmosphere. The reaction solution was then passed through a H$^+$ - saturated Dowex MSC-1 cation exchange column, dialyzed in a 1000 MWCO membrane, and freeze dried. The enzyme-catalyzed reaction was performed similarly, with

the addition of 100 mg HRP and 16 mL hydrogen peroxide, with a reaction time of 11 days.

Preparation of compost samples for NMR analysis

All compost samples were air-dried, passed through a 710-micron sieve, and desiccated prior to solid-state NMR analysis. The portions of DNT composts analyzed by NMR were also soxhlet extracted with methanol prior to analysis.

NMR spectrometry

Liquid-phase ^{15}N NMR spectra were recorded on a GEMINI 2000 NMR spectrometer at a nitrogen resonant frequency of 30.4 MHz using a 10-mm broad-band probe. The spectrum of the methanol extract from the 2,4-D^{15}NT compost, dissolved in DMSO-d$_6$ with Cr(acac)$_3$, was acquired using a 23,649.6-Hz (778-ppm) spectral window, 45-deg pulse angle, 0.5-s acquisition time, 1.0-s pulse delay, and inverse gated decoupling. Spectra of humic and fulvic acids were recorded using the ACOUSTIC sequence (Patt 1982). Spectra of the humic acids reacted with the 4M3NA were recorded on approximately 300 mg of sample dissolved in DMSO-d$_6$, with addition of ~80 mg of the relaxation reagent Cr(Acac)$_3$. Spectra of the fulvic and humic acids extracted from the 2,4-D^{15}NT compost were recorded on 150 mg or less of sample dissolved in ^{13}C-depleted DMSO-d$_6$ with an appropriate amount of Cr(acac)$_3$. Acquisition parameters for the ACOUSTIC sequence included an 18,656.7-Hz (613.7-ppm) spectral window, 0.2-s acquisition time, 45-deg pulse angle, 0.5-s pulse delay, and τ delay of 0.1 ms. Neat formamide in a 5-mm NMR tube, assumed to be 112.4 ppm, was used as an external reference standard for all spectra. The ^{15}N NMR chemical shifts are reported in ppm downfield of ammonia, taken as 0.0 ppm. Solid-state CP/MAS ^{15}N NMR spectra were recorded on a Chemagnetics CMX-200 NMR spectrometer at a nitrogen resonant frequency of 20.3 MHz using a 7.5-mm ceramic probe (zirconium pencil rotors). Acquisition parameters for ^{15}N included a 30,000-Hz spectral window, 17.051-ms acquisition time, 5.0-ms contact time (2.0 ms for ^{15}NH$_4$^{15}NO$_3$ compost), 0.5-s pulse delay, and spinning rate of 5 KHz. Nitrogen-15 chemical shifts were referenced to glycine, taken as 32.6 ppm.

Quantitation in NMR spectra

With the use of paramagnetic relaxation reagent, liquid-phase ^{15}N NMR spectra recorded using the ACOUSTIC sequence should represent the quantitative distribution of nitrogens incorporated into the humic and fulvic acids. In solid-state CP/MAS experiments, peak areas can accurately represent the number of nuclei resonating, when the time constant for cross-polarization is significantly less than the time constant for proton spin lattice relaxation in the rotating frame, $T_{NH} \ll T_{1\rho}H$. Since no analyses of the spin dynamics were performed, or a comparison made with direct polarization experiments, the solid-state CP/MAS spectra can be interpreted only semiquantitatively. A more detailed comparison of solid-state CP/MAS, direct polarization mass/magic angle spinning

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(DP/MAS), and liquid-state ^{15}N NMR spectra of TNT compost fractions was presented in Thorn, Pennington, and Hayes (2002).

3   Results

Interactions with Soils and Compost

Adsorption kinetics

Both 2,4- and 2,6-DNT reached steady-state partitioning in the low organic carbon soil, Grange Hall, almost immediately (Figures 3 and 4). However, 2,6-DNT required 3 days (75 hr) to reach steady state in the Picatinny soil; 2,4-DNT did not reach steady state in this soil in 5 days (120 hr). Both compounds failed to reach steady state during 5 days in the high organic carbon soil, Yokena Clay. These results indicate that the compounds are continuing to be removed from the solution phase. Some of the removal may be due to transformation and subsequent partitioning of the transformation products. However, the appearance of the monoamino transformation products and their subsequent decline are consistent with covalent bonding to soil organic carbon. The formation of diamino transformation products, which were not observed, requires more reducing conditions than are likely to be achievable in these tests. Removal of both 2,4- and 2,6-DNT from the solution phase was greatest in the soil highest in TOC, CEC and clay (Yokena soil).

Partition coefficients

Partition coefficients for the DNTs were relatively low and roughly comparable to those reported for TNT and RDX in the same or comparable soils (Pennington and Patrick 1990; Brannon et al. accepted for publication). Coefficients for 2,4-DNT were significantly (95 percent confidence interval) higher than those for 2,6-DNT (Figure 5). These results suggest at least some soil sorption potential for these compounds. However, the partition coefficients for 2,4-DNT in Picatinny soil and for both 2,4- and 2,6-DNT in Yokena Clay soil, both of which are relatively high in TOC, are only estimates, since steady-state adsorption was not achieved during the 120-hr kinetics test (Figure 4). Failure to achieve steady state may have been due to transformation followed by covalent binding to functional groups on the organic carbon in these soils.
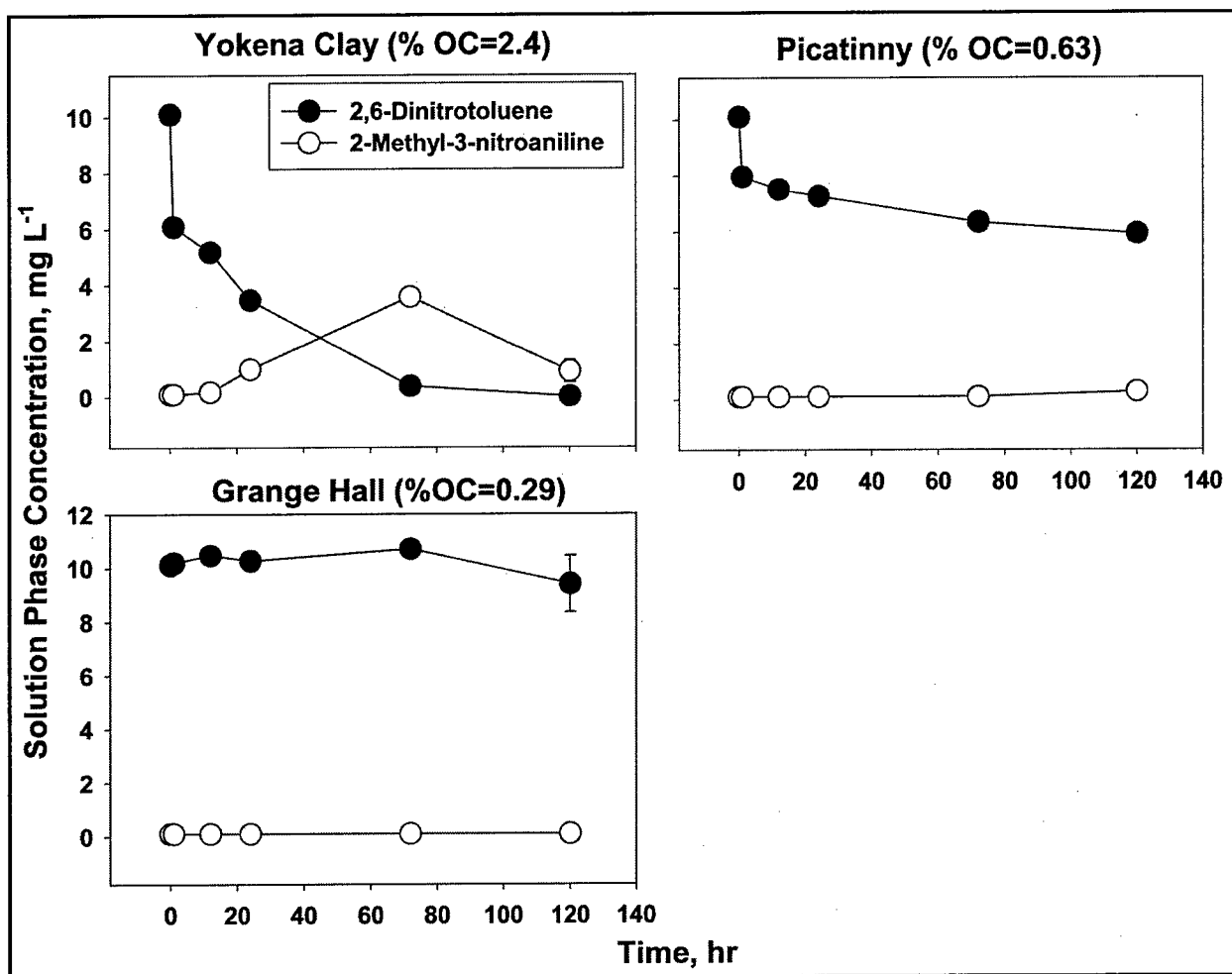
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Figure 3. Adsorption kinetics for 2,6-DNT on three soils having different organic carbon content. Data points are means of three replicates ± one standard deviation unit (error bars are typically too small to show up). The monoaminonitrotoluene transformation product, 2-methyl-3-nitroaniline (2M3NA, the only monoamine possible) appears, then nearly disappears in the highest organic carbon soil, Yokena Clay. Other soils yielded little (Picatinny) or no (Grange Hall) 2M3NA. The diamino transformation product was not detected

Sequential desorption and desorption kinetics

Negligible amounts of 2,4-DNT (0.06 ± 0.01 µg mL^{-1}) were desorbed from the Fort Lewis soil in the three sequential desorption cycles, and the small amounts in each cycle did not differ significantly from each other ($p < 0.05$). Therefore, no desorption coefficient was determined for this field-contaminated soil. No analytes other than the low concentrations of 2,4-DNT were observed in the solution phase of the tests. Similarly, no 2,4-DNT nor related compounds were detected in the solution phase of desorption kinetics tests. Therefore, since no desorption occurred, determination of desorption kinetics was not relevant.
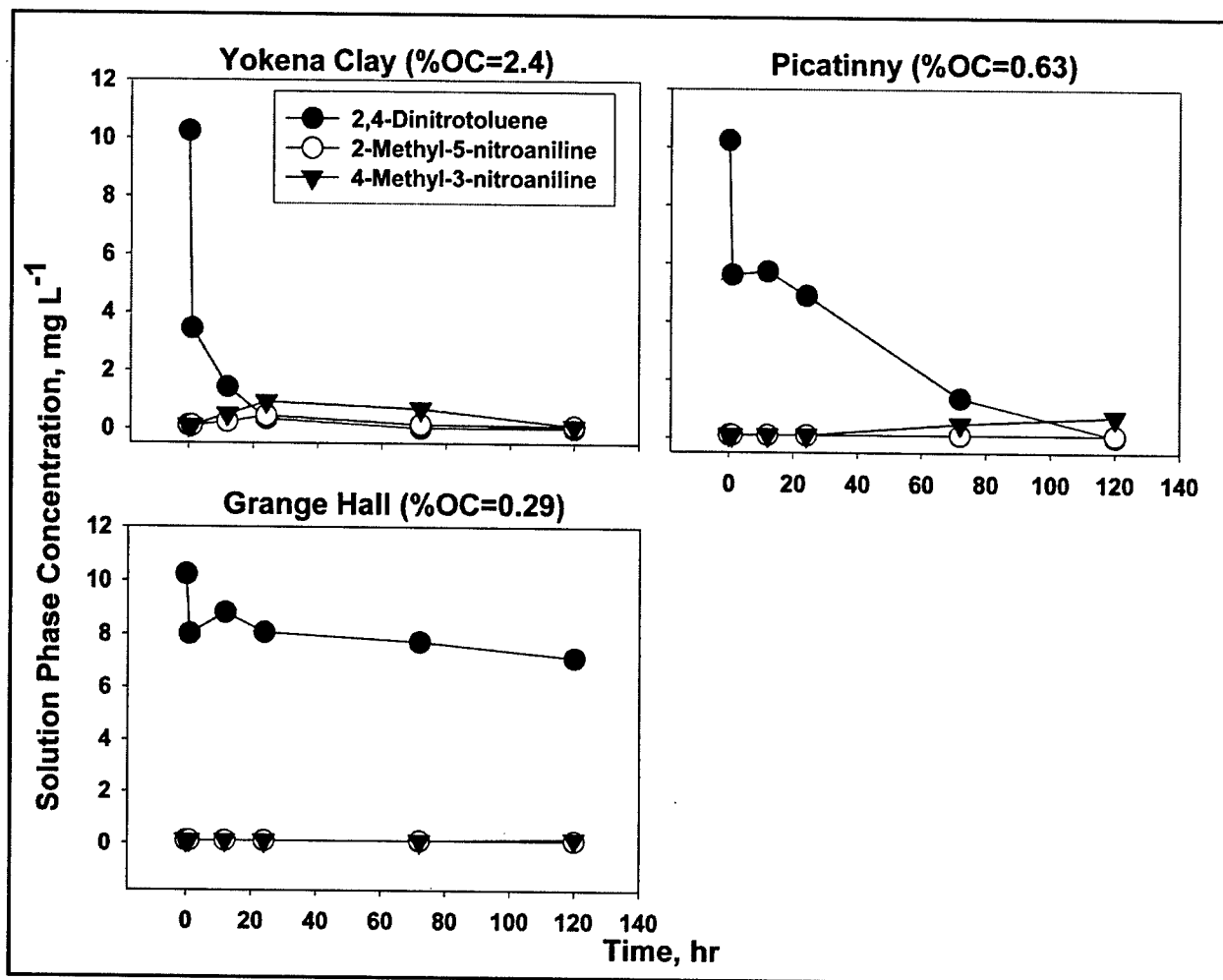
Figure 4. Adsorption kinetics for 2,4-DNT on three soils having different organic carbon content. Data points are means of three replicates ± one standard deviation unit (error bars are typically too small to show up). The monoaminonitrotoluene transformation products, 2-methyl-5-nitroaniline and 4-methyl-3-nitroaniline, appear in both Yokena Clay and Picatinny soils, but then disappear in the Yokena Clay. The diaminotoluene transformation product was not observed

Soil fractionation

Soil extraction with acetonitrile produced the greatest recovery of radio-activity, an indication that a significant portion of the added compounds had not reacted with the soil (Table 2). Although total recoveries for the 2,4-DNT were poor (only 49.13 percent compared with 82.27 percent for 2,6-DNT), results indicated at least limited association of 2,4-DNT with organic fractions of the soil in a form that was unextractable with acetonitrile. Therefore, at least a small portion of the added 2,4-DNT was bound to the matrix. A significant portion of the 2,6-DNT was associated with the fulvic acid fraction. While the radioactivity recovered from other fractions did not differ, association of 2,6-DNT with the various fractions was measurable and significant. Nearly 50 percent of the added radioactivity was associated with unextractable fractions. These results indicate that both 2,4- and 2,6-DNT are bound to the soil organic matrix to some extent.
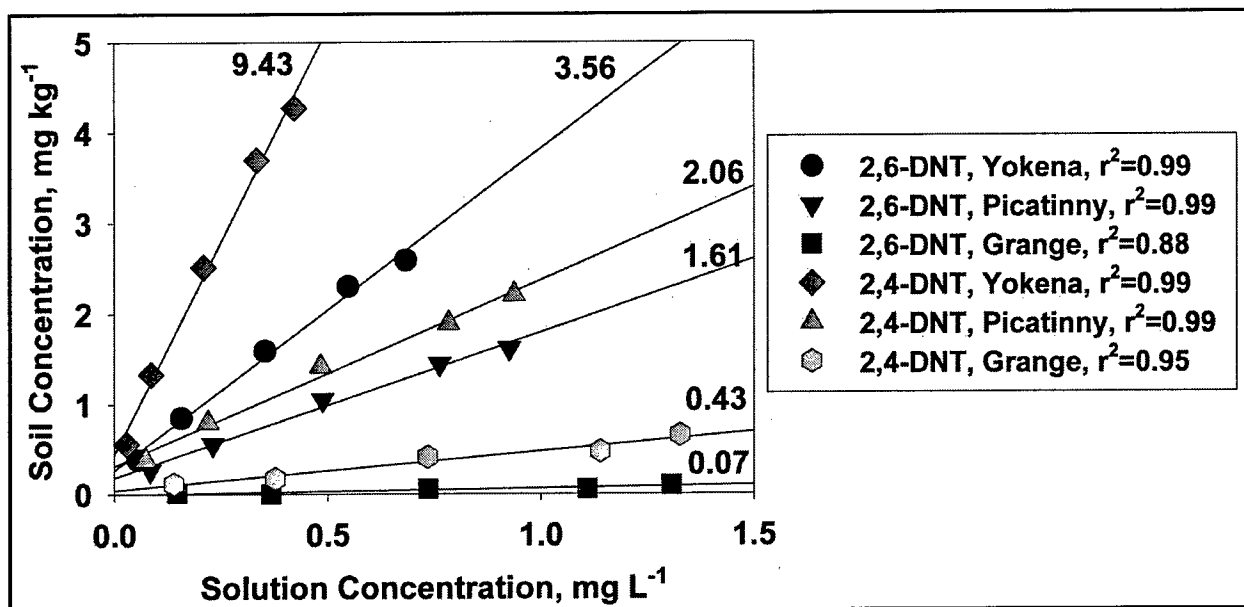
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Figure 5. The soil partition coefficients K_ds for 2,4- and 2,6-DNT in three soils are given by the slope indicated on the regression line. The coefficients for 2,4-DNT in Picatinny soil, and for both 2,4- and 2,6-DNT in Yokena Clay soils are only estimates, since steady-state adsorption was not achieved during the 120-hr kinetics test (Figure 4)

Table 2
Percent Radioactivity Recovered from Each Fraction of Soil after 20 Days of Composting

Replicate	Acetonitrile	Fulvic Acid	Cellulose	Humin	Humic Acid
2,4-DNT					
1	40.65	0.66	1.15	0.085	0.040
2	43.82	0.72	1.76	0.11	0.062
3	55.83	0.80	1.47	0.15	0.076
Mean	46.77[1]	0.73	1.46	0.11	0.059
Standard Deviation	8.00	0.07	0.31	0.034	0.018
2,6-DNT					
1	31.32	21.96	9.00	2.71	1.51
2	30.68	29.74	10.13	3.43	1.39
3	38.87	42.24	16.43	5.36	2.06
Mean	33.62*	31.31*	11.85	3.83	1.65
Standard Deviation	5.56	10.23	4.00	1.37	0.36
[1] Significantly greater ($p < 0.05$) than other fractions of that compound.					

Composts

Compost mass balance

Recoveries of added radioactivity from compost at the initiation of the test by combustion of two subsamples from each of the seven replicates were 100 percent (means ± standard deviations were 119.95 ± 24.31 percent for 2,4-DNT

reactors; 94.22 ± 10.05 percent for 2,6-DNT reactors). As indicated by these standard deviations the initial matrix was still highly heterogeneous even after extensive mixing. Mass balance of ^{14}C radioactivity after 20 days of composting indicated that no VOCs and only low levels of CO_2 were generated. Total radioactivity recovered as CO_2 for 2,4-DNT reactors was 1.55 ± 0.30 percent and for 2,6-DNT reactors was 0.78 ± 0.28 percent. Results of fractionation of the finished compost at 20 days indicated that most of the remaining radioactivity was associated with the cellulose fraction (Table 3). The 2,4- and 2,6-DNT composts exhibited similar proportions of most fractions. Less than 5 percent of the added radioactivity was recovered by acetonitrile extraction. This is the fraction used in chemical analysis by HPLC. No analytes related to the DNTs were identified by HPLC analysis of the acetonitrile extracts.

Table 3 Radioactivity Recovered from Each Fraction as a Percent of the Total Activity in Whole Compost after 20 Days					
Replicate	Acetonitrile	Fulvic Acid	Cellulose	Humin[1]	Humic Acid
2,4-DNT					
1	0.51	1.12	15.28	1.24	0.41
2	0.47	1.14	18.18	1.06	0.25
3	0.38	1.05	15.38	0.98	0.35
4	0.37	1.37	16.11	-	0.57
5	0.48	1.45	15.15	-	0.59
6	0.46	1.53	15.75	-	0.57
Sum	2.68	7.66	96.85	3.28	2.74
Mean	0.45	1.28	16.14^2	1.09	0.46
Standard Deviation	0.06	0.20	1.10	0.13	0.14
2,6-DNT					
1	0.78	1.28	9.92	0.73	0.74
2	0.79	1.48	9.46	0.70	0.88
3	0.87	1.21	0.69	-	0.97
4	0.74	1.25	8.01	-	0.97
5	0.60	1.31	7.69	-	0.75
6	0.95	1.31	9.29	-	1.04
Sum	4.73	7.84	54.06	1.43	5.35
Mean	0.79	1.31	9.01^2	0.72	0.89
Standard Deviation	0.12	0.093	0.93	0.021	0.12
[1] Insufficient humin remained in each replicate for analysis. Therefore, replicates were combined to generate three samples for 2,4-DNT and two samples for 2,6-DNT assays. [2] Significantly greater ($p < 0.05$) than other fractions of that compound.					

Compost of ^{15}N-labeled compounds

Analysis of the 2,4-D^{15}NT compost at 20 days by HPLC revealed significant decreases over initial concentrations (Table 4). Without a radioactive tracer, determining whether this decrease resulted from degradation or unextractable immobilization cannot be discerned. However, results of NMR analyses will reveal whether bound products are present.

Table 4
Results of HPLC Analyses of ^{15}N-Labeled Compounds after Composting for 20 Days

Replicate μg g^{-1}	2,4-DNT[1]	2,6-DNT	Nitrobenzene	Aniline
1	12,400	5,260	153	1,260
2	11,000	4,900	434	1,390
Mean	11,700	5,800	293	1,325
Standard Deviation	990	255	199	91.9
% of added	21.96	14.88	1.61	4.13

[1] 2,710 ± 566 μg g^{-1} 4M3NA was also detected in the 2,4-DNT treatment.

Summary

Results of soil interaction studies demonstrated that both 2,4- and 2,6-DNT undergo transformation in soils and interact with soil by mechanisms that are less reversible than soil adsorption. These interactions are amplified in the high organic carbon matrix of compost where less than 5 percent of the radioactivity was recovered by solvent extraction. Clearly, the association of DNTs with this organic matrix constitutes a significant environmental fate for these compounds. An understanding of the nature and mechanism of these reactions provides an indication of the potential for long-term stability of the products of these reactions and is the subject of the NMR analyses presented in the following section.

NMR Analyses

Reaction of soil humic acid with 4M3NA

As a guide to the interpretation of the liquid-phase ^{15}N NMR spectra of 4M3NA reacted with the soil humic acid, background data from previous studies are reproduced here. Reactions of aromatic amines with organic functional groups are summarized in Figure 6, and a compilation of ^{15}N NMR chemical shifts of nitrogen functional groups relevant to these studies in Figure 7. Assignments for ^{15}N NMR spectra of aniline reacted with humic substances are shown in Table 5. Nitrogen-15 NMR chemical shifts of the parent DNTs and reduction products are listed in Figure 8. The ability of 4M3NA to undergo covalent binding with soil humic acid in the absence of any catalyst is confirmed in the quantitative liquid-phase ^{15}N NMR spectra in Figure 9. Major peaks occur at 84, 109, 120, 137, 172, 182, and 308 ppm. The chemical shift of the unreacted 4M3NA occurs at 61 ppm. The peak at 377 ppm corresponds to the naturally abundant ^{15}N nuclei in the unlabeled nitro groups of the 4M3NA molecules covalently bonded to the humic acid. From these data, the assignments can be made as listed in Table 6.
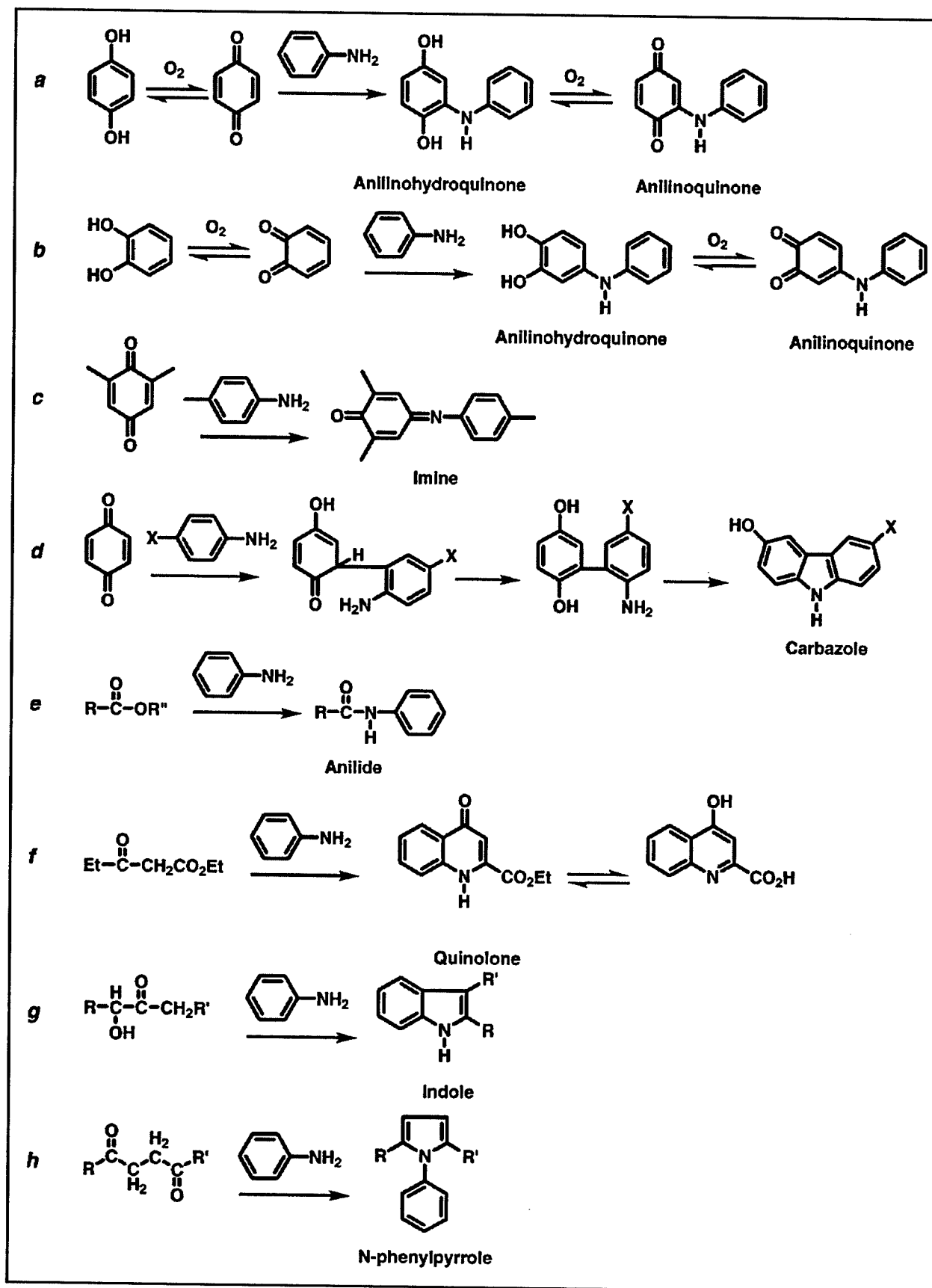
Figure 6. Summary of reactions between aromatic amines and organic functional groups (Sheet 1 of 3)
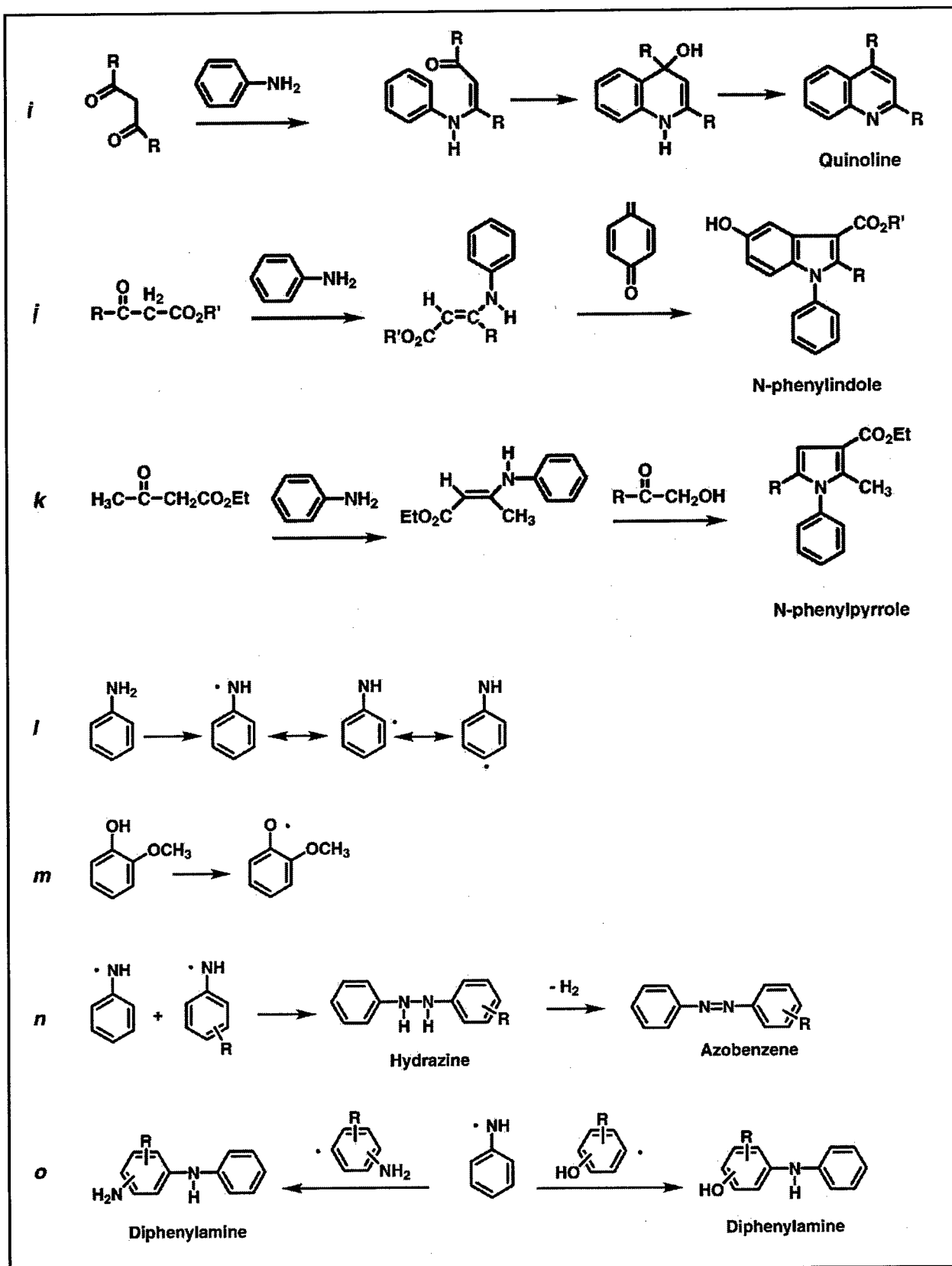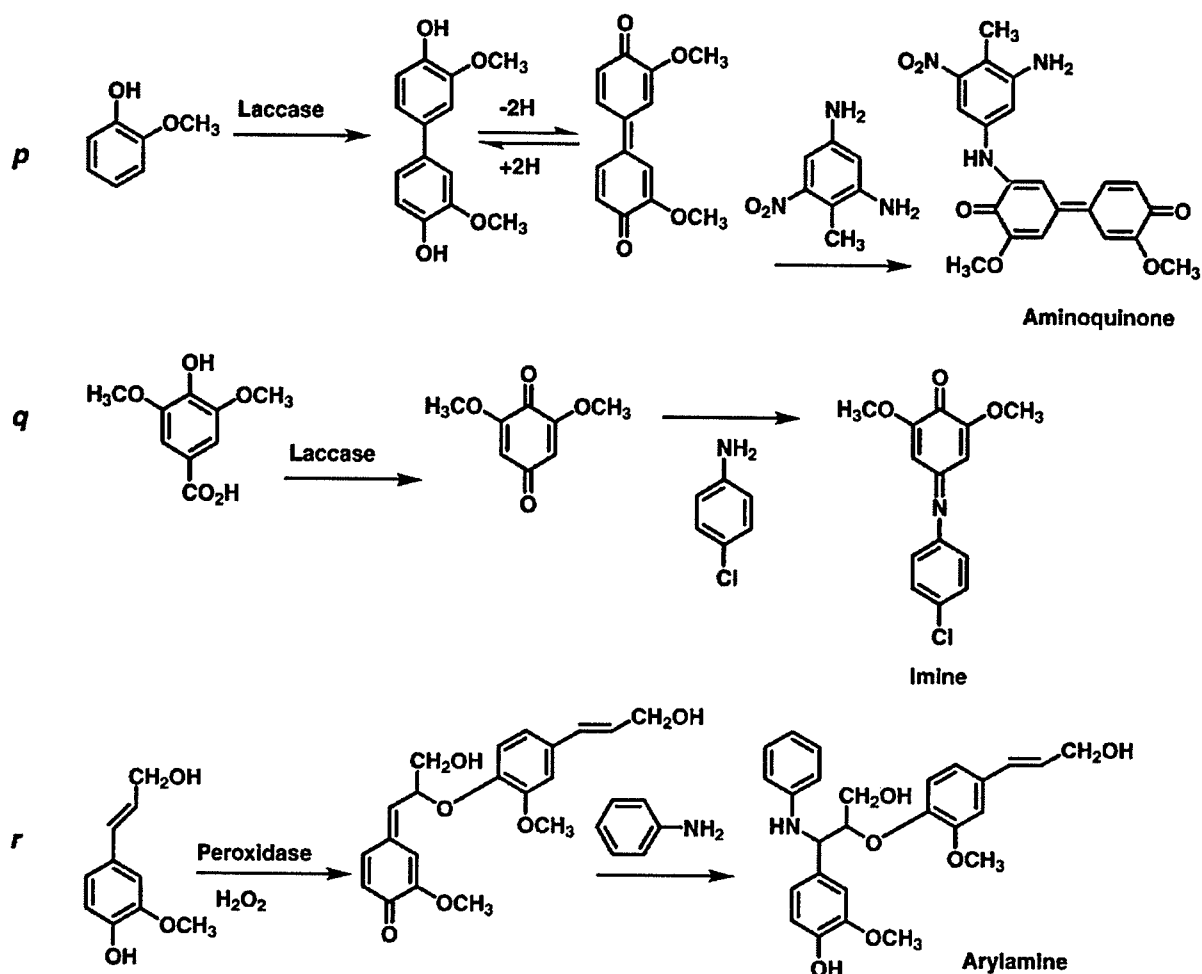
Figure 6.   (Sheet 2 of 3)

The following table of citations lists the sources of these reactions:

Reactions of Aromatic Amines with Organic Functional Groups

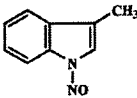| Reactions	Reference	Synthesis Name
a-d	(Kutyrev 1991)	
c	(Ononye and Graveel 1994)	
f	(Newkome and Paudler 1982)	
g	(Brown 1972)	Knorr
h	(Newkome and Paudler 1982)	Bischler Indole
i	(Joule, Mills, and Smith 1995)	
j	(Brown 1972)	Nenitzescu
k	(Newkome and Paudler 1982)	Hantzsch
l	(Peter 1989; Monks et al. 1992; Naidja, Huang, and Bollag 2000; Dec and Bollag 2000)	
m	(Peter 1989; Monks et al. 1992; Naidja, Huang, and Bollag 2000; Dec and Bollag 2000)	
n	(Peter 1989; Monks et al. 1992; Naidja, Huang, and Bollag 2000; Dec and Bollag 2000)	
o	(Peter 1989; Monks et al. 1992; Naidja, Huang, and Bollag 2000; Dec and Bollag 2000)	
p	(Dawel et al. 1997)	
q	(Bollag, Minard, and Liu 1983)	
r	(Lange, Hertkorn, and Sandermann 1998)	

Figure 6.   (Sheet 3 of 3)
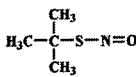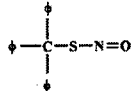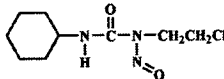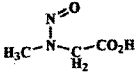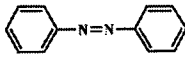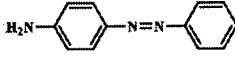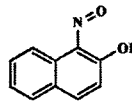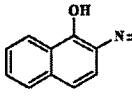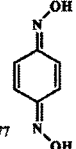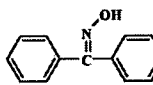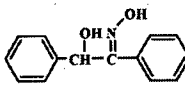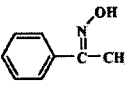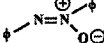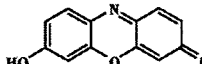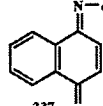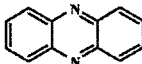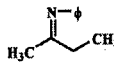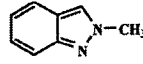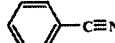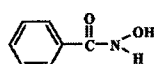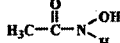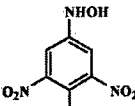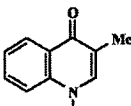
Nitrosobenzene [a]   Thionitrite [a]   Thionitrite [a]   Sodium Nitrite [a]   Nitrous Acid [a]   N-nitrosamine

913   834   807   610   562   555

N-nitrosamide [a]   N-nitrosamine [a]   Azobenzene [a]   Azo-hydrazone [a]

562   E=540, Z=534   511   501   473

Nitrosonaphthol   Nitrosonaphthol   Sodium Nitrate [a]   Nitric Acid [a]   Quinone Dioxime   Nitrobenzene [a]

425   418   378   378 - 351   377   372

(a)

Oxime   Oxime   Oxime   Azoxybenzene [a]   Azoxybenzene [a]   Phenoxazinone [c]

362   357   356, 325   360   344   333   326   338

Quinone Imine [b]   Imine [a]   Phenazine [a]   Imine [a]   Quinoline   Pyrazole [a]

337   330   328   323   315   288   218

Nitrile   Nitrile   Oxazole   Imidazole [a]   Imidazole [a]   Hydroxamic Acid

256   243   249   244   144   209   166

Hydroxamic Acid   Pyrrole   Phenylhydroxylamine   Phenylhydroxylamine [c]   Quinolone [a]   Indole [a]

166   158   137   132   134   133

(b)

Figure 7.   Nitrogen-15 NMR chemical shifts of nitrogen functional groups. Chemical shifts determined in DMSO-d_6 in the USGS lab unless otherwise noted. (a) from references (Levy and Lichter 1979; Martin, Martin, and Gouesnard 1981; Witanowski, Stefaniak, and Webb 1986, 1993; Berger, Braun, and Kalinowski 1997) (b) tentative assignment (c) determined in solid state (Continued)

Anilide Anilide Enaminone [b] Enaminone [b] Carbazole [a]

132 130 127 114 114

Anilinoquinone Anilinoquinone Anilinoquinone Hydroxylamine [a] 1° Amide 1° Amide

112 103 102 109 105 107

Hydroxylamine HCl [a] Aminoquinone Phenoxazine Anilinohydroquinone Anilinohydroquinone

NH₂OH.HCl

86 85 77 74 73

Hydrazine Aminodiphenylamine Aniline Aminophenol Ammonium Chloride [a]

NH₄Cl

95 87 58 58 47 25-30

(c)

Figure 7. (Concluded)

Table 5
Summary of Assignments for ^{15}N NMR Spectra of Samples Reacted with ^{15}N-Labeled Aniline

Chemical Shift Range, ppm	Assignment [1]
Noncatalyzed Reactions	
60-100	*anilinohydroquinone*, phenoxazine
100-122	*anilinoquinone*, carbazole, enamine
122-148	*anilide*, enaminone, quinolone, indole
148-200	N-phenylindole, N-phenylpyrrole, *heterocyclic N*
300-350	*imine*, phenoxazinone, quinoline
Catalyzed Reactions (Peroxidase, Tyrosinase, Birnessite) [2]	
60-100	diphenylamine, arylamine, hydrazine
230-280	imidazole, pyrazole, oxazole, nitrile
310-360	*imine*, iminodiphenoquinone, azoxybenzene
470-525	*azobenzene*

[1] Most probable assignments indicated by italics.
[2] For samples reacted with aniline in the presence of peroxidase, tyrosinase, and birnessite, assignments from both sections apply.
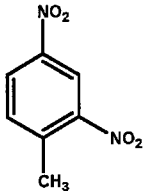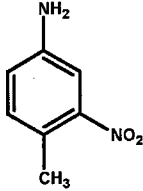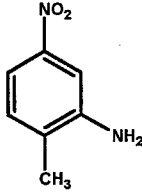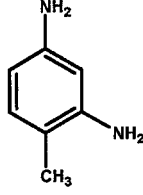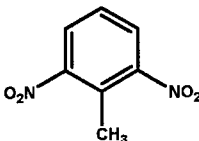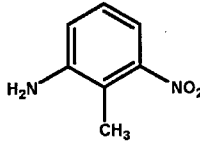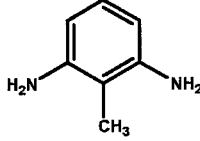
2,4-Dinitrotoluene

$\delta^{15}N$ = 370.3 ppm (l)
    365.9 ppm (l)

$\delta^{15}N$ = 377 ppm (s)
    368 ppm (s)

4-Amino-2-nitrotoluene

(4-Methyl-3-nitroaniline)

$\delta^{15}N$ = 377.9 ppm (l)
    61.1 ppm (l)

2-Amino-4-nitrotoluene

(2-Methyl-5-nitroaniline)

$\delta^{15}N$ = 372.8 ppm (l)
    63.4 ppm (l)

2,4-Diaminotoluene

$\delta^{15}N$ = 57.2 ppm (l)
    56.3 ppm (l)

$\delta^{15}N$ = 57 ppm (s)

2,6-Dinitrotoluene

$\delta^{15}N$ = 372.4 ppm (l)

$\delta^{15}N$ = 370 ppm (s)

2-Amino-6-nitrotoluene

(2-Methyl-3-nitroaniline)

$\delta^{15}N$ = 379.8 ppm (l)
    63.5 ppm (l)

$\delta^{15}N$ = 381 ppm (s)
    59 ppm (s)

2,6-Diaminotoluene

$\delta^{15}N$ = 57.7 ppm (l)

$\delta^{15}N$ = 55 ppm (s)

Figure 8. Liquid and solid-state ^{15}N NMR chemical shifts of DNTs and amines, l = liquid, s = solid

Interestingly, the signal-to-noise ratio obtained in the spectrum is superior to that of spectra recorded on the humic acid reacted with the monoamines, 2ADNT and 4ADNT, using comparable concentrations of sample and acquisition times. This indicates that 4M3NA is more reactive than 2ADNT or 4ADNT with respect to nucleophilic addition to carbonyl groups. Peaks corresponding to heterocyclic (172 ppm, 182 ppm) and imine (308 ppm) nitrogens are more clearly resolved in the 4M3NA spectrum than in the corresponding 2ADNT and 4ADNT spectra. Peak areas as a percent of total nitrogen for quantitative liquid phase ACOUSTIC ^{15}N NMR spectra of IHSS soil humic acid reacted with 4M3NA are shown in Table 7.

Phenol oxidase enzymes (peroxidases, tyrosinases, and laccases) and metal catalysts (oxides or oxyhydroxides of aluminum, iron, and manganese) promote the one-electron oxidation of contaminant aromatic amines and aromatic moieties within humic substances (Naidja, Huang, and Bollag 2000; Dec and Bollag 2000) (reactions *l* and *m*, Figure 6).

4M3NA Std
LB = 1 Hz

61

4M3NA /Soil HA
LB = 70 Hz

182 172
137
120
109
84
61
377
308

4M3NA /Soil HA/HRP
LB = 50 Hz

154 138 25
171
183
105
86
372
309 254

600   500   400   300   200   100   0
ppm
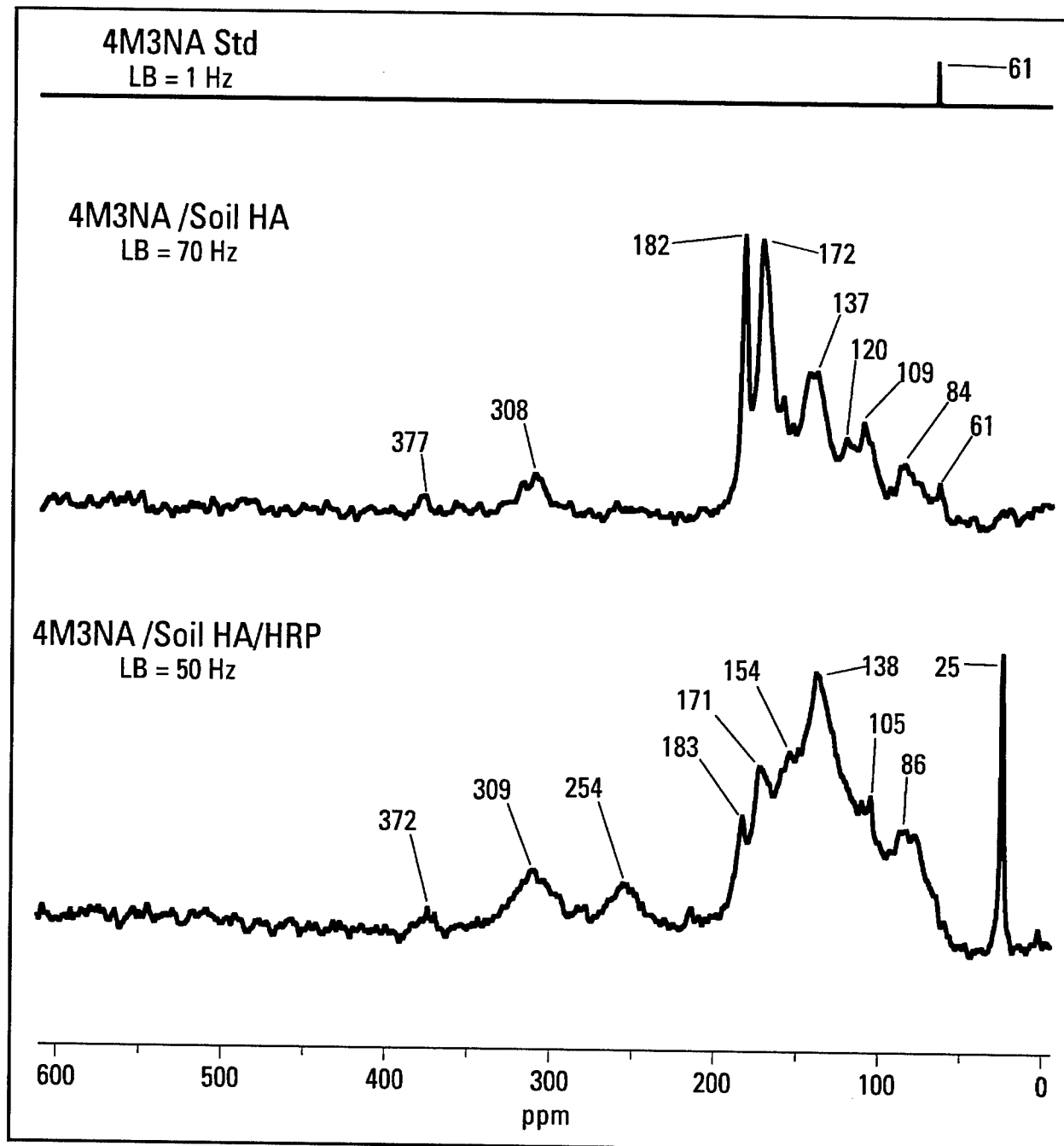
Figure 9. Quantitative liquid-phase ACOUSTIC ^{15}N NMR spectra of IHSS soil humic acid reacted with 4M3NA with and without HRP as catalyst. Liquid-phase ^{15}N NMR spectrum of 4M3NA. HA = humic acid; LB = line broadening

The catalysts can increase the amount of covalent binding by effecting free radical coupling reactions between aromatic amines and the humic molecules, or by creating additional substrate sites within the humic molecules for subsequent nucleophilic addition by the amines. Nitrogen to N free radical couplings to form hydrazine and azobenzene adducts (reaction *n,* Figure 6) and C to N couplings to form diphenylamine structures (reaction *o,* Figure 6) are therefore possible under conditions of catalysis. Numerous examples of the creation of substrate sites in

Table 6
Assignments for ^{15}N Spectra of Soil Humic Acid Reacted with 4M3NA

Chemical Shift, ppm	Assignment
61	residual 4M3NA
84	aminohydroquinone
109	aminoquinone
137	amide
172	heterocyclic
182	heterocyclic
308	imine
377	unlabeled nitro

Table 7
Peak Areas as Percent of Total Nitrogen for Quantitative Liquid Phase ACOUSTIC ^{15}N NMR Spectra of IHSS Soil Humic Acid Reacted with 4M3NA-^{15}N [1]

Sample	390-360 ppm	360-275 ppm	275-220 ppm	220-140 ppm	140-40 ppm	40-0 ppm ammonia
HA/4M3NA	1	8	1	54	36	0
HA/4M3NA/ HRP	2	13	8	34	40	4

[1] Electronic integration.

reactions with model lignin compounds have been reported in the literature. These include the laccase mediated dimerization of guaiacol to the diphenoquinone followed by nucleophilic addition of 2,4DANT to form the anilinoquinone structure (Dawel et al. 1997) (reaction *p*, Figure 6); the laccase catalyzed oxidative decarboxylation of syringic acid to the hindered 2,6-dimethoxy-1,4-benzoquinone followed by 1,2-addition of 4-chloroaniline to produce the imine (Bollag, Minard, and Liu 1983) (reaction *q*, Figure 6); the peroxidase catalyzed coupling of coniferyl alcohol to the β aryl ether structure followed by condensation with aniline to form the arylamine (Lange, Hertkorn, and Sandermann 1998) (reaction *r*, Figure 6).

Several effects of HRP on the reaction of 4M3NA with soil humic acid are evident from the NMR spectrum (Figure 9): (a) a shift away from heterocyclic nitrogen formation (peaks at 172 and 182 ppm) concomitant with an increase in imine formation (309 ppm); (b) formation of the nitrogens at 254 ppm, tentatively assigned as imidazole, pyrazole, or oxazole; and (c) release of ammonia (peak at 25 ppm) from deamination of 4M3NA. These effects reproduce almost exactly the effects of HRP on the covalent binding of the diamines 2,4DANT and 2,6DANT with the soil humic acid reported previously (Thorn and Kennedy 2002). As discussed previously, catalysts increase the number of potential pathways for covalent binding reactions (Thorn and Kennedy 2002; Thorn et al. 1996). Certain types of covalent bonds formed only with phenoloxidase enzyme or metal catalysis may overlap in terms of chemical shift with bonds formed in the absence of catalysts, complicating the task of assigning peaks. Thus, for

example, in spectra of HRP- and birnessite-catalyzed reactions, hydrazine (~95 ppm), arylamine (~80 ppm), and diphenylamine (~87 ppm) nitrogens may overlap with aminoquinone and aminohydroquinone nitrogens in the region from approximately 70 to 100 ppm.

The change in peak intensities in the region upfield of 220 ppm compared with those of the noncatalyzed reaction may thus reflect a contribution from three sets of reactions: (a) oxidative decarboxylation of para-hydroxybenzene carboxylic acids to quinones followed by 1,4 addition of 4M3NA to form aminohydroquinone and aminoquinone adducts; (b) nitrogen to nitrogen coupling to form hydrazines; (c) nitrogen to carbon coupling to form diphenylamine nitrogens.

Liquid-state ^{15}N NMR spectrum of 2,4-D^{15}NT compost extract

A portion of the dried composts were soxhlet extracted with methanol prior to recording of solid-state CP/MAS ^{15}N NMR spectra. A significant amount of color came off both the 2,4- and 2,6-D^{15}NT composts. This was in contrast to the T^{15}NT compost experiments, in which very little color was extracted. The liquid-state ^{15}N NMR spectrum of the 2,4-D^{15}NT methanol extract indicates a mixture of unreduced 2,4-D^{15}NT and 2,4-D^{15}NT reduction products (Figure 10). The main constituent appears to be 4M3NA (peaks at 60.7 and 375.8 ppm). Peaks at 368.9 and 364.6 ppm are assumed to be 2,4-D^{15}NT, and the peaks at 371.5 ppm and 62.8 ppm are assumed to be 2M5NA. The peak at 615.5 ppm most likely corresponds to a nitroso compound, possibly 2-nitroso-4-nitrotoluene or 4-nitroso-2-nitrotoluene. Liu, Thomson, and Anderson (1984) detected these nitroso intermediates in *anaerobic* fermentations of 2,4-DNT. The diamine reduction product 2,4-diaminotoluene was not detected in the ^{15}N NMR spectrum. The liquid-state spectrum of the methanol extract of the 2,6-D^{15}NT compost (not shown) indicated the presence of the parent 2,6-D^{15}NT, monoamine reduction product 2M3NA, and an unidentified nitroso intermediate. The diamine reduction product 2,6-diaminotoluene was not detected.

Solid-state CP/MAS ^{15}N NMR spectra of whole composts

Solid-state CP/MAS ^{15}N NMR spectra of the whole composts of 2,4- and 2,6-D^{15}NT are compared with spectra of T^{15}NT, nitrobenzene-^{15}N, aniline-^{15}N, and ^{15}NH$_4$^{15}NO$_3$ composts in Figure 11. Vertical scale expansions of the DNT spectra are shown in Figure 12. As discussed previously, the CP/MAS spectra can be interpreted only semiquantitatively, as nitro, imine, and heterocyclic nitrogens may be underestimated.

The composts of 2,4-DNT, 2,6-DNT, and TNT were exhaustively solvent extracted. The resonances observed in the ^{15}N NMR spectra are therefore interpreted as corresponding to the labeled nitrogens of the reduced amines covalently bonded to organic matter. The spectra of the 2,4- and 2,6-DNT composts are very similar to one another, and, in general, are consistent with covalent bonding by the monoamine reduced degradation products of the DNTs to organic matter. The peaks at 377 ppm (2,4-DNT) and 378 ppm (2,6-DNT) correspond to the nitro groups of the aminotoluenes covalently bonded to organic matter through amine

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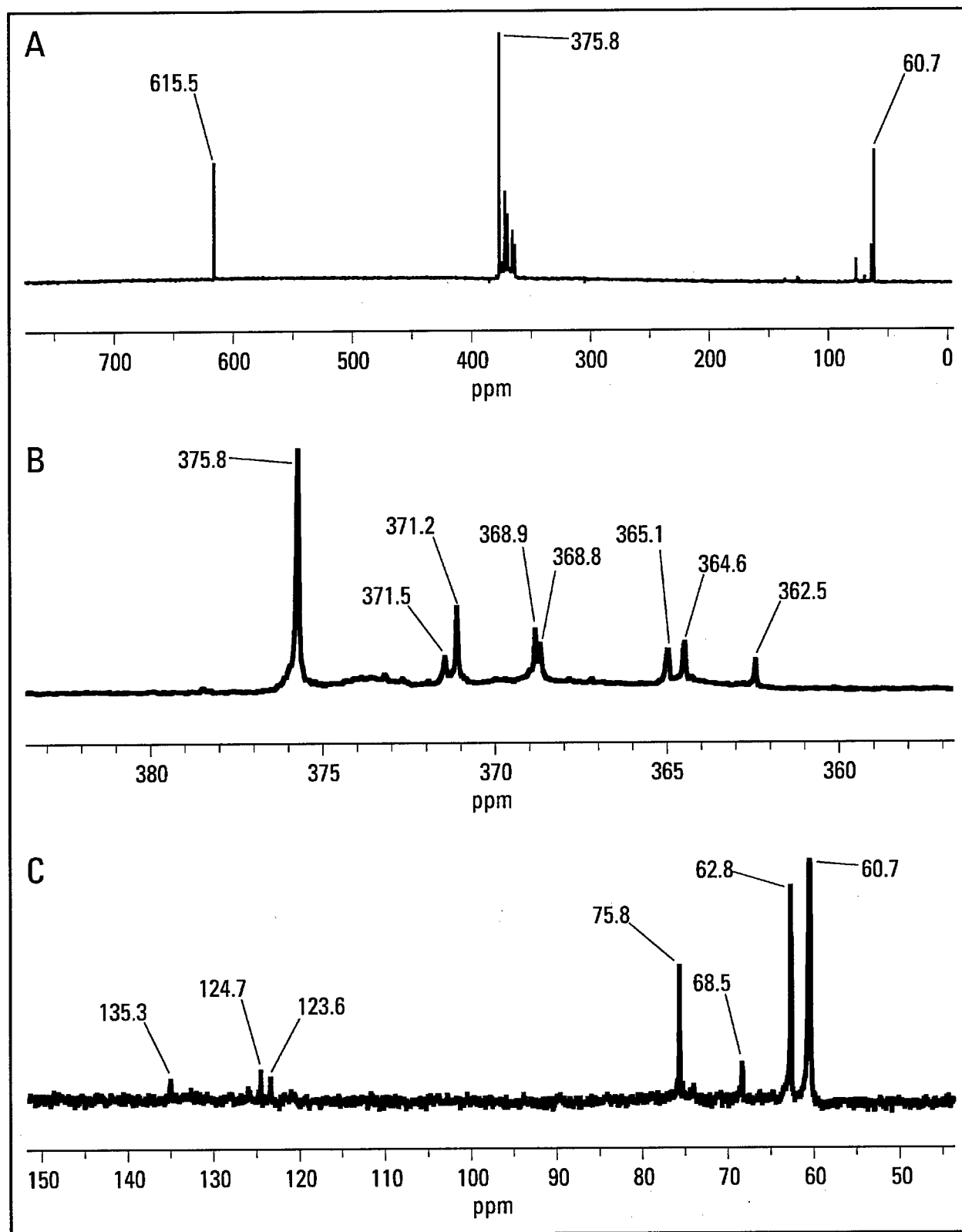
Figure 10. Liquid-phase inverse gated decoupled ^{15}N NMR spectrum of methanol extract of 2,4-D^{15}NT compost
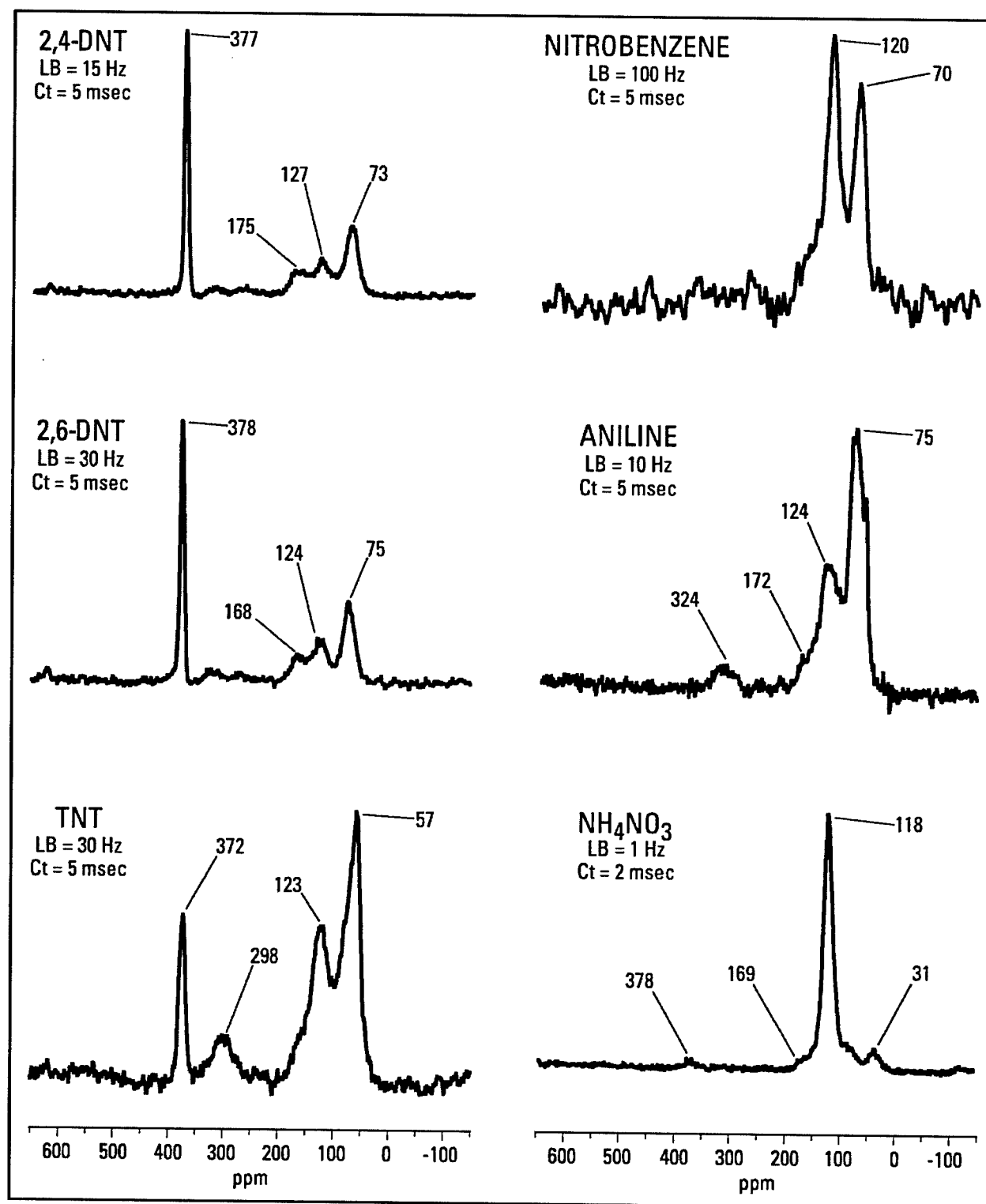
Figure 11. Solid-state CP/MAS ^{15}N NMR spectra of whole composts of 2,4-D^{15}NT, 2,6-D^{15}NT, T^{15}NT, nitrobenzene-^{15}N, aniline-^{15}N, and $^{15}NH_4$$^{15}NO_3$. The composts of 2,4-D^{15}NT, 2,6-D^{15}NT, T^{15}NT were solvent extracted prior to NMR acquisition. LB = line broadening in Hertz; Ct = contact time in msec
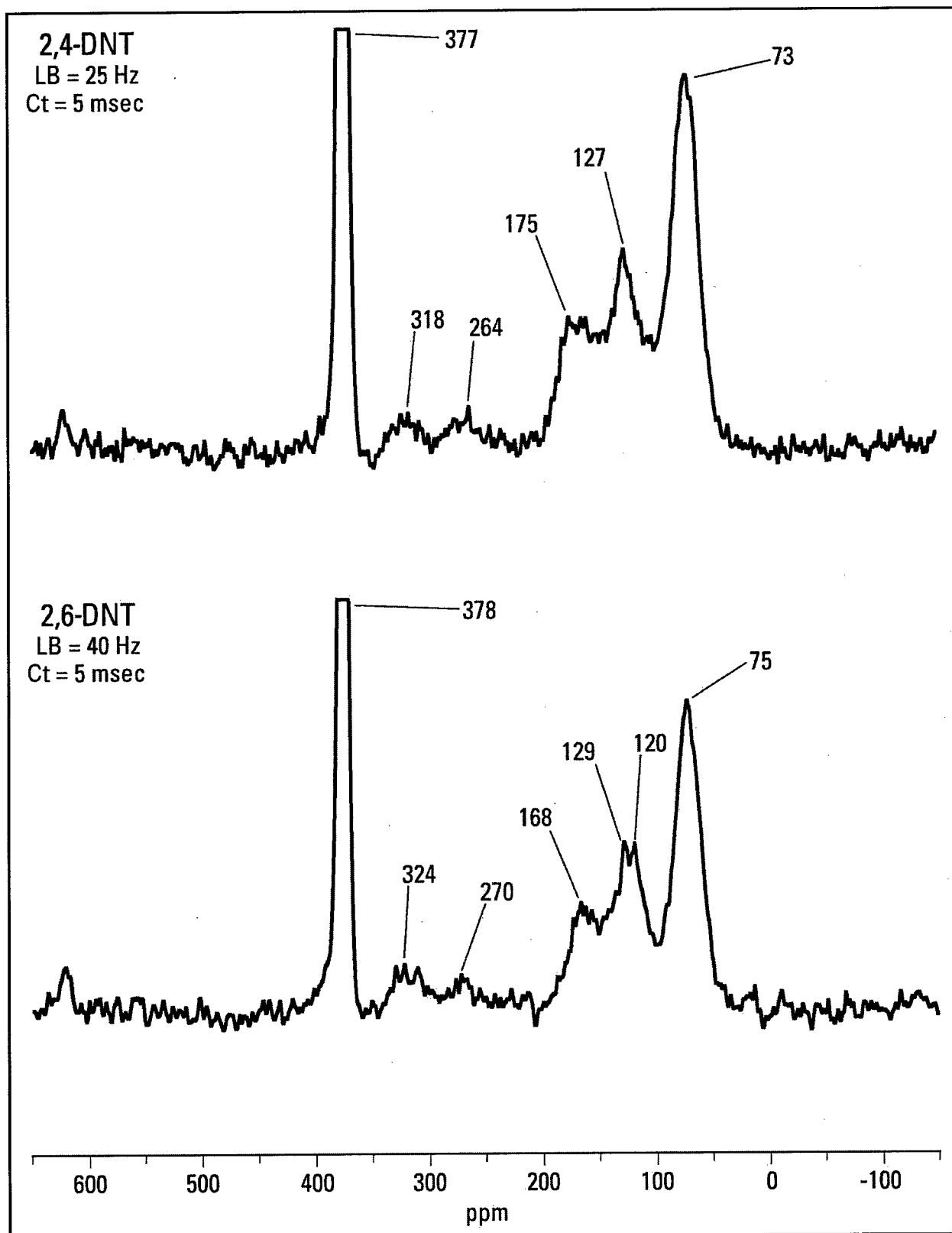
Figure 12. Vertical scale expansion of solid-state CP/MAS ^{15}N NMR spectra of whole composts of 2,4-D^{15}NT and 2,6-D^{15}NT

groups. The peaks upfield of approximately 350 ppm correspond to the various bonds formed between the amine groups of the aminonitrotoluenes and the carbonyl groups of the organic matter. The well-defined peaks of the DNT spectra can be assigned as shown in Table 8.

Table 8 Assignment of ^{15}N NMR Spectra of Composts of 2,4-D^{15}NT and 2,6-D^{15}NT	
Chemical Shift Range, ppm	Assignment
0 – 100	aminohydroquinone, phenoxazine, diphenylamine, arylamine, hydrazine
100 – 150	aminoquinone, carbazole, enamine, amide, enaminone, quinolone, indole
150 – 200	N-phenylindole, N-phenylpyrrole, heterocyclic N
200 – 360	imine, phenoxazinone, quinoline, imidazole, pyrazole, oxazole nitrile, iminodiphenoquinone, azoxybenzene
360 – 390	nitro

A comparison of the spectra of the DNTs, TNT, and nitrobenzene shows the relative degree of reduction of the nitroaromatics that occurs during the aerobic composting. The absence of nitro groups in the spectrum of the nitrobenzene compost indicates complete reduction of nitrobenzene, followed presumably by covalent binding of the resulting aniline to the organic matter. Partial mineralization of the nitrobenzene to CO_2 and ammonia cannot be ruled out (see last paragraph of this section). The ratio of nitro to reduced nitrogens is greater in the DNT spectra than in the TNT spectrum. This is consistent with the fact that under aerobic conditions reduction of one of the two nitro groups of the DNTs is possible, whereas reduction of two of the three nitro groups of TNT is possible.

Two other differences between the DNT and TNT spectra are notable. The concentration of imine nitrogens is greater in the TNT spectrum. This may be explained by the fact that, because TNT is readily reduced to the diamines, 2,4DANT and 2,6DANT, and the diamines have a greater propensity to undergo 1,2-addition to quinone groups to form imines than the monoamines, the potential for imine formation is greater for TNT than the DNTs. The chemical shift positions of the upfield peak maxima occur at 73 ppm and 75 ppm in the spectra of the DNTs and at 57 ppm in the spectrum of TNT. In the TNT compost, the free amine groups (indicated by asterisk in Figure 13) of diamine molecules covalently bonded to organic matter through one amine group give rise to the peaks at ~ 57 to 60 ppm. The peak maxima at 73 and 75 ppm in the DNT spectra correspond primarily to the nitrogens of the aminohydroquinone and arylamine adducts of the aminonitrotoluenes.

Vertical scale expansion of the D^{15}NT compost spectra reveals peaks at 264 ppm (2,4-D^{15}NT) and 270 ppm (2,6-D^{15}NT). These peaks correspond to imidazole, pyrazole, or oxazole nitrogens. The occurrence of these nitrogens may constitute evidence for the action of phenoloxidase enzymes in covalent binding of the amines to the organic matter.

Figure 13. Free amine groups of diamine molecules covalently bonded to organic matter through one amine group. Free amine groups indicated by asterisks

The nitrogens observed in the ^{15}N NMR spectra of the D^{15}NT and T^{15}NT composts represent the nitrogens of the reduced D^{15}NT and T^{15}NT metabolites covalently bonded to organic matter. In contrast, ^{15}NH$_4$^{15}NO$_3$ added to the compost mixture and subjected to the 21-day aerobic incubation is taken up by fungi and bacteria in the microcosm and converted into protein and other bio-chemical constituents. The subsequent humification pathways of the biologically immobilized nitrogen upon expiration of the microbial biomass are poorly under-stood (Clinton, Newman, and Allen 1995; Cheshire et al. 1999). The solid-state CP/MAS ^{15}N NMR spectrum of the ^{15}NH$_4$^{15}NO$_3$ amended compost illustrates the alternative pathway of biological immobilization of nitrogen (Figure 11). The major peak at 118 ppm corresponds to secondary amide nitrogens in peptide structures; the peak at 31 ppm, amino sugars and free amino groups of amino acids. The shoulder downfield of the secondary amide nitrogens, including the peak at 169 ppm, may include heterocyclic sp^3 hybridized nitrogens such as indoles, pyrroles, and the imide or lactam nitrogens of nucleosides. The two resonances corresponding to the starting ^{15}NH$_4$^{15}NO$_3$ at 375 ppm and 22 ppm have almost completely disappeared, with only a minor nitrate peak still visible at 378 ppm.

In contrast to TNT and the DNTs, aniline has the potential to undergo mineralization to carbon dioxide and ammonia under aerobic conditions (Rieger and Knackmuss 1995). If aniline were mineralized during the composting, a spectrum resembling that of the ^{15}NH$_4$^{15}NO$_3$ compost would be expected. The spectrum of the aniline compost, however, is consistent mainly with covalent binding of aniline to organic matter of the compost, and bears a strong resem-blance to solid-state CP/MAS ^{15}N NMR spectra of aniline reacted with whole soil, peat, and soil humic acid (Thorn et al. 1996). A likely explanation is that the aniline underwent covalent binding to organic matter immediately upon addition to the compost and prior to the start of the incubation, and that the covalently bound aniline was resistant to mineralization during incubation. The spectrum of the nitrobenzene compost appears to be consistent with reduction of the

nitrobenzene to aniline followed by covalent binding of the aniline to organic matter. In both the aniline and nitrobenzene composts, the occurrence of mineralization and immobilization cannot be completely ruled out from the NMR analyses alone. The problem of spectral overlap precludes complete resolution between the processes of covalent binding by aniline on one hand and mineralization/immobilization on the other. Peptide nitrogens (118 ppm) resulting from mineralization/immobilization can overlap with nitrogens at 120-124 ppm corresponding to aniline covalently bound to organic matter through anilinoquinone and anilide linkages.

Solid-state CP/MAS ^{15}N NMR spectra of 2,4-D^{15}NT compost fractions

Lignocellulose, humin, fulvic acid, and humic acid fractions were isolated from the 2,4-D^{15}NT compost. Lignocellulose composed approximately 90 percent of the compost organic matter. Solid-state CP/MAS ^{15}N NMR spectra of these fractions are shown in Figure 14. In general, the same resonances present in the spectrum of the whole 2,4-DNT compost are also evident in the spectra of the fractions. The overall similarity of the spectra of the individual fractions suggests that the monoamine degradation products of 2,4-DNT form similar types of bonds to the organic matter of all fractions. One interesting feature in the spectrum of the humin fraction is the splitting of the upfield peak into separate resonances at 56 ppm and 73 ppm. The resonance at 55 ppm may correspond to the free amine group (indicated in Figure 15 by asterisks) of 2,4-diaminotoluene covalently bonded to organic matter through one amine group.

If so, this would constitute evidence for reduction of the 2,4-DNT to the diamine stage. Consistent with this observation is the fact that the ratio of nitro to reduced nitrogens (measured by peak areas, Table 9) in the humin spectrum is less than in the other fractions.

Liquid-phase ^{15}N NMR spectra of humic and fulvic acid fractions

A comparison of the quantitative liquid-phase (Figure 16) versus solid-state CP/MAS ^{15}N NMR spectra (Figure 14) of the fulvic and humic acid fractions from the 2,4-D^{15}NT compost indicates that nitro and imine nitrogens are under-estimated in the CP/MAS experiment. For example, nitro groups compose 29-36 percent of the total nitrogen in the CP/MAS spectra and 65-66 percent of the total nitrogen in the ACOUSTIC spectra (Tables 9 and 10). The CP/MAS experiment was also found to underestimate nitro, imine, and heterocyclic nitrogens in the humin and fulvic acid fractions extracted from the T^{15}NT compost (Thorn, Pennington, and Hayes 2002). These results provide a specific illustration of the problems in quantitation that should be considered in the application of the solid-state cross-polarization experiment to soil materials.
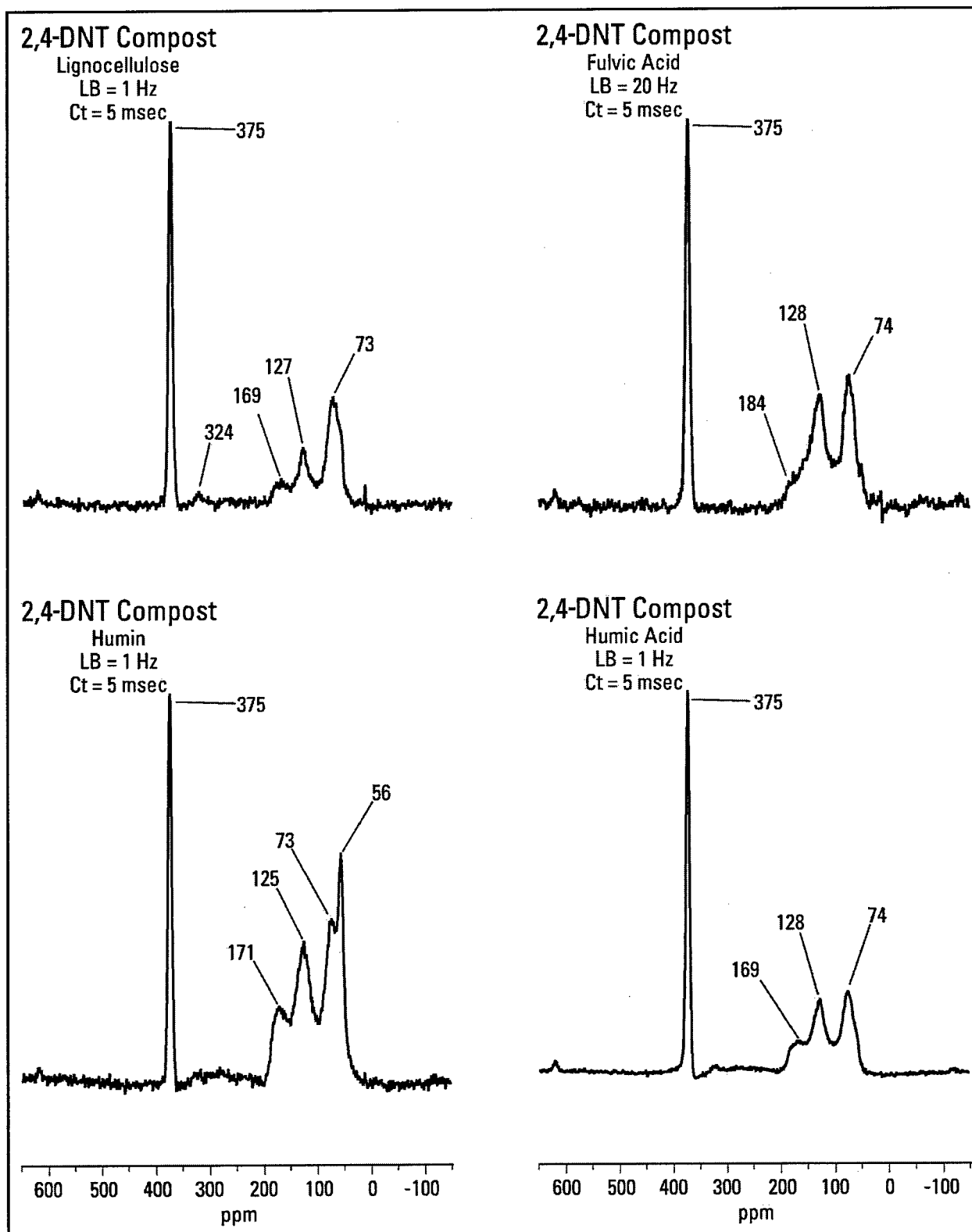
Figure 14. Solid-state CP/MAS ^{15}N NMR spectra of lignocellulose, humin, humic acid, and fulvic acid fractions isolated from whole compost of 2,4-D^{15}NT
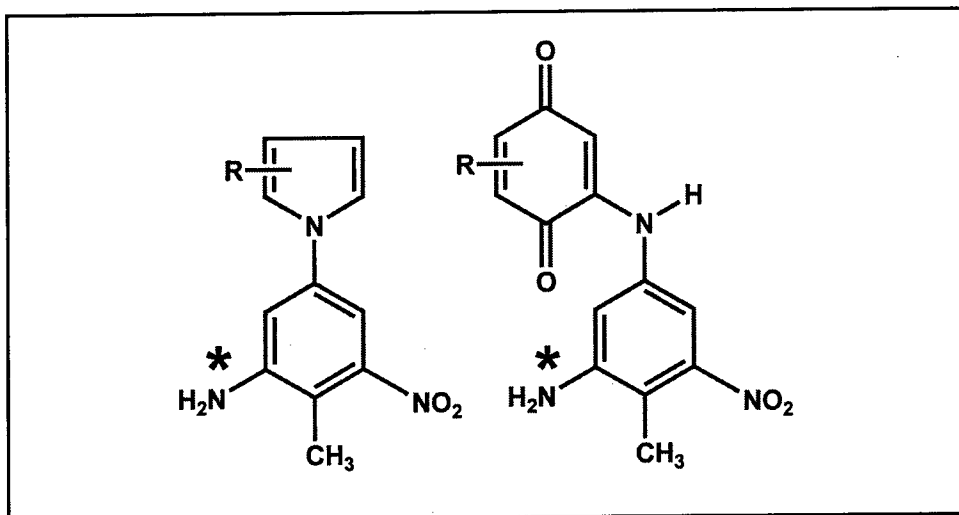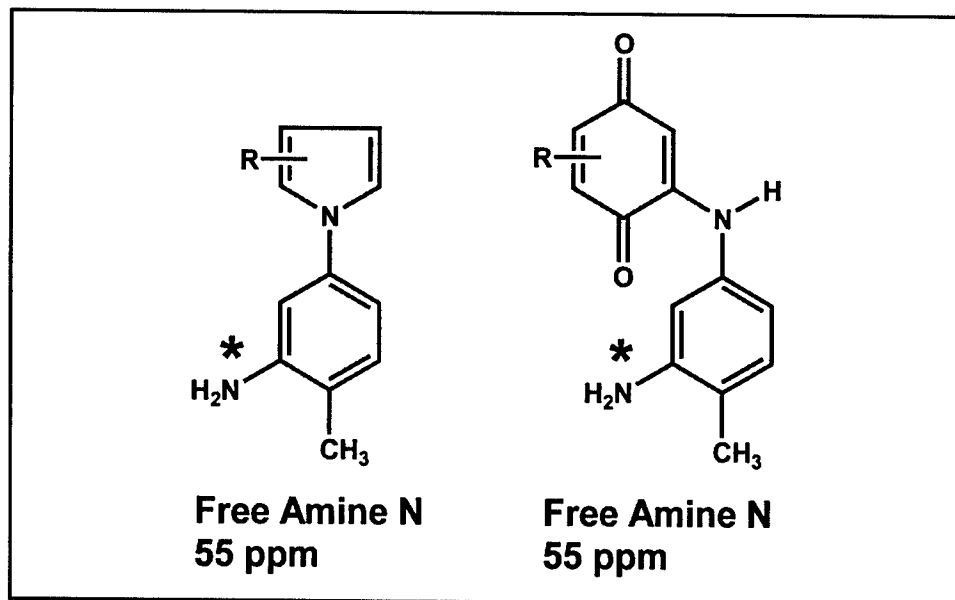
Figure 15. Free amine group of 2,4-diaminotoluene covalently bonded to organic matter through one amine group. Free amine group indicated by asterisk

Table 9
Peak Areas as Percent of Total Nitrogen for Solid State CP/MAS ^{15}N NMR Spectra of Composts and 2,4-DNT Compost Fractions [1]

Sample	390-360 ppm	360-200 ppm	200-150 ppm	150-100 ppm	100-0 ppm
TNT					
2,6-DNT	32	10	9	18	30
2,4-DNT	35	4	11	18	32
2,4-DNT Lignocellulose	36	6	8	16	34
2,4-DNT Humin	18	0	13	27	43
2,4-DNT Humic Acid	36	1	10	25	29
2,4-DNT Fulvic Acid	29	1	10	27	33

[1] Peak areas are semiquantitative in CP/MAS experiments.
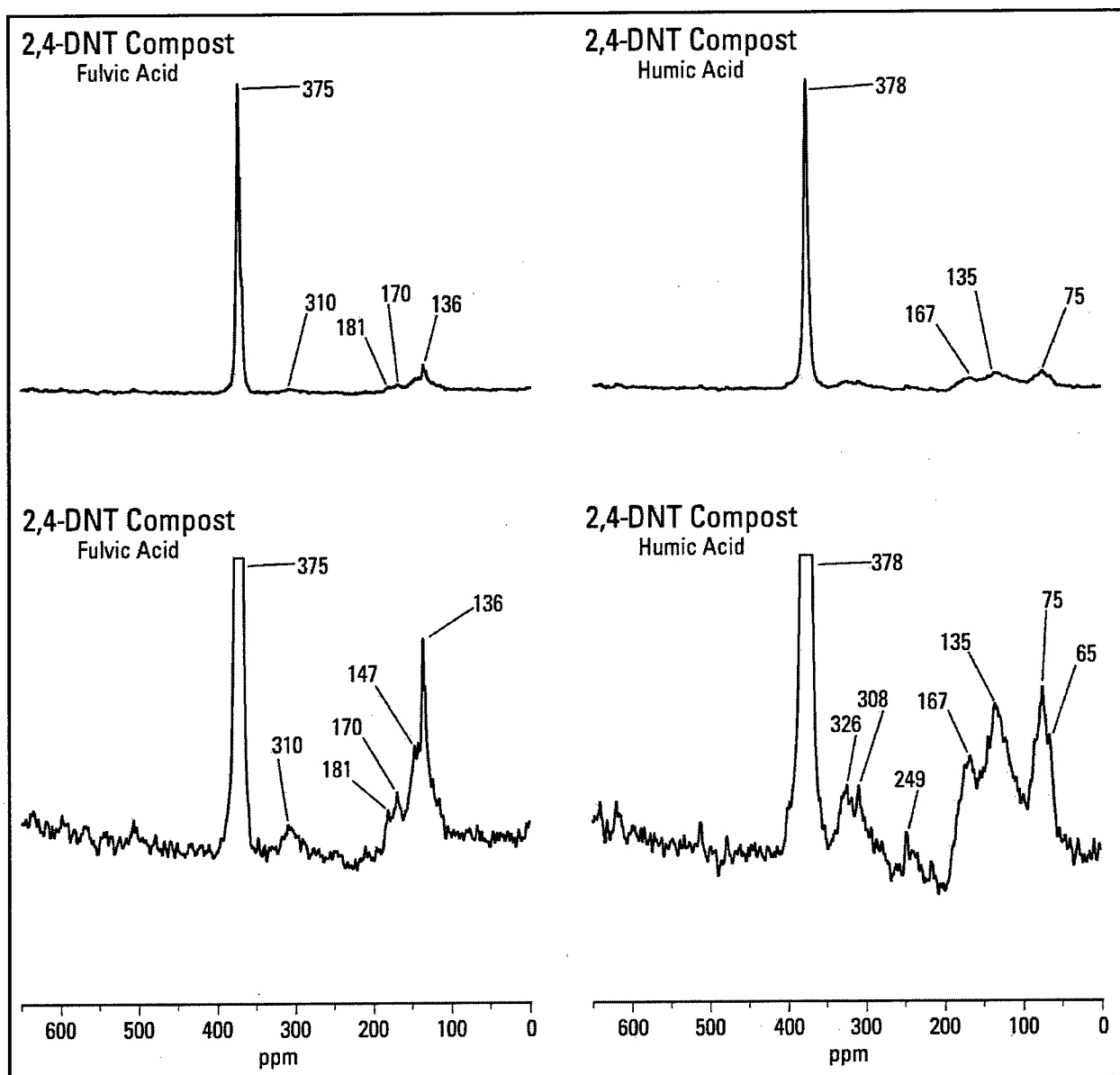
Figure 16. Quantitative liquid-phase ACOUSTIC ^{15}N NMR spectra of humic and fulvic acid fractions isolated from whole compost of 2,4-D[15]NT

Table 10
Peak Areas as Percent of Total Nitrogen for Quantitative Liquid-Phase ACOUSTIC ^{15}N NMR Spectra of Fulvic and Humic Acids Isolated from 2,4-D[15]NT Compost [1]

Sample	390-350 ppm	350-268 ppm	268-200 ppm	200-150 ppm	150-0 ppm
Fulvic Acid	66	5	0	8	21
Humic Acid	65	6	0	6	25
[1] Electronic integration.					

4 Conclusions

The ^{15}N NMR analyses have confirmed that during aerobic composting, DNTs are reduced to monoaminonitrotoluenes that subsequently undergo covalent bonding to organic matter. The similarity of the solid-state CP/MAS ^{15}N NMR spectra of the 2,4-D^{15}NT and 2,6-D^{15}NT composts indicates a similar distribution of the types of covalent bonds formed between the reduced metabolites and the functional groups of the organic matter. In the case of the 2,4-D^{15}NT compost, an examination of the humin fraction revealed evidence for reduction of 2,4-D^{15}NT to the diamines. Lignocellulose material comprises the bulk of organic matter in the 2,4-DNT compost (~90 percent). The majority of reduced 2,4-D^{15}NT metabolites are covalently bonded to the lignocellulose material. This also is presumed to be the case in the 2,6-DNT compost, although it was not fractionated. The occurrence of peaks at 264-270 ppm (imidazole, pyrazole, oxazole nitrogens) in the spectra of the whole composts may be taken as evidence for the action of phenoloxidase enzymes in the covalent bonding of the amines to organic matter. The amount of solvent-extractable reduced and unreduced DNTs from the finished composts suggests that the aerobic incubation experiments were less efficient for the DNTs than for TNT, both in terms of the percentages of substrates reduced and percentages of reduced metabolites incorporated into organic matter through covalent bonds.

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14. ABSTRACT

Covalent bonding of amino transformation products of trinitrotoluene (TNT) to functional groups on humic acid results in immobilized products that are not hydrolyzable, microbially degradable, or leachable. However, the extent to which these reactions occur with dinitrotoluenes (DNTs) was unknown. Since DNTs are considered toxic and many explosives-contaminated sites exhibit DNTs as well as TNT, the fate of DNTs is relevant to remediation and risk assessment. The broad objectives of this study were to demonstrate the potential for immobilization reactions of DNTs in soils and to determine the mechanisms of bonding of amino transformation products of DNTs to humic acid and humin in soils and compost treatment systems. DNTs were partitioned to soils having different physical characteristics to define adsorption and desorption kinetics and partitioning coefficients. Radiolabeled [^{14}C]DNTs were used to amend soils prior to composting so that mass balance of the organic fractions of the compost could be determined and any volatile products indicated. Finally, ^{15}N-labeled DNTs were used to amend soils prior to composting so that organic fractions could be analyzed by nuclear magnetic resonance (NMR) spectrometry. The fulvic acid, humic acid, humin, and lignocellulose fractions isolated from the 2,4-DNT compost were also analyzed by ^{15}N NMR. Aniline, nitrobenzene, and ammonium nitrate, all labeled with ^{15}N, were also subjected to the aerobic composting.

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Steady-state partitioning was not achieved in high organic carbon soils, an indication that the compounds continue to be removed from the solution phase, perhaps due to transformation and subsequent partitioning/reaction of the transformation products with the organic carbon fractions of the soil. Mass balance of ^{14}C radioactivity during composting indicated that no volatile organic compounds (VOCs) and barely detectable levels of CO_2 were generated. Most of the radioactivity was associated with the cellulose fraction.

Results of the ^{15}N NMR analyses confirmed that during aerobic composting DNTs are reduced to monoamino-nitrotoluenes that subsequently undergo covalent bonding to organic matter. The similarity of the solid-state cross-polarization/magic angle spinning (CP/MAS) ^{15}N NMR spectra of the 2,4-D^{15}NT and 2,6-D^{15}NT composts indicated a similar distribution of the types of covalent bonds formed between the reduced metabolites and the functional groups of the organic matter. Most of the reduced 2,4-D^{15}NT metabolites were covalently bonded to the ligno-cellulose material. This also is presumed to be the case in the 2,6-DNT compost, although it was not fractionated. The occurrence of peaks at 264-270 ppm (imidazole, pyrazole, oxazole nitrogens) in the spectra of the whole composts provided evidence for the action of phenoloxidase enzymes in the covalent bonding of the amines to organic matter. The amount of solvent-extractable reduced and unreduced DNTs from the finished composts suggested that the aerobic incubation experiments were less efficient for the DNTs than for TNT, both in terms of the percentages of substrates reduced and percentages of reduced metabolites incorporated into organic matter through covalent bonds.

Results of this study confirmed the transformation and subsequent covalent bonding of DNTs with soil and compost organic carbon, defined the kinds of bonds produced, and provided a comparison between reactions of TNT and the DNTs.